CONTENTS

JANUARY, NO. 1

iii

iv   CONTENTS

APRIL, NO. 4

MAY, NO. 5

JUNE, NO. 6

CONTENTS

DECEMBER, NO. 12

THE MALE HORMONE CONTENT OF RUMINANT MANURE[1]

C. W. TURNER

Department of Dairy Husbandry, University of Missouri, Columbia

While the bull testis served as the source of the first extract of male hormone (8, 9) and bull urine was found to be one of the paths of excretion of slight amounts of this hormone (7), it was not until 1942 that Riley and Hammond presented evidence indicating that dairy cattle during certain physiological states excrete considerable amounts of male hormone by way of the digestive tract in the manure. They noted the precocious development of the comb and wattles of chicks fed dried cow manure as part of the starter ration without stimulation of the gonads. This observation not only indicated the presence of some androgenic hormone in the manure but its oral availability to the chick as well.

The largest content of androgens was observed to be present in the manure of dairy cows in lactation in various stages of pregnancy. Manure from heifers not pregnant produced slight comb development, whereas that from bulls was reported to be without effect.

The secretion of considerable amounts of male hormone by the lactating cow and its elimination in the feces adds significance to the observations of many investigators that androgens stimulate the growth of the mammary glands and may play a rôle in stimulating the lactation process. It seemed of interest, therefore, to study further the extent of elimination of the male hormone by various ruminants preliminary to a detailed study of the excretion rate of this hormone during various physiologic periods.

EXPERIMENTAL[2]

The method of assay of the androgenic hormone in the manure of various species of ruminants has been essentially the same as that described by Riley and Hammond (11). White Plymouth Rock chicks were substituted for Rhode Island Reds. The basal chick starter is the same as used in our

1

previous studies (14) except that the alfalfa meal was eliminated in proportion to the addition of manure up to 10 per cent of the diet. At the end of 4 weeks, the combs were removed even with the surface of the head and weighed. The average comb weight and the comb weight per 100 gm. body weight indicates the extent of androgen stimulation. When the chicks were sexed, the sum of the average weight of the combs of the males and females was divided by two.

Since David (2) showed that the sensitivity of the capon comb to androgen stimulation showed a seasonal variation of as much as 100 per cent from the peak response in winter to the minimum in the summer, the date of each assay is reported. It might be expected that a similar seasonal variation would occur in our assay method since a seasonal rhythm in thyroid secretion rate of chicks has been observed (10) under the same environmental conditions.

The urine-free manure was collected daily, placed in a thin layer in an electric drying oven, and heated to dryness at a temperature of 45° C. or slightly less, except where especially indicated. Usually about 48 hours were required to dry this material. The manure was then ground in a mill to the consistency of alfalfa meal. The starter ration containing the dried cow manure in amounts up to 20 per cent was eaten without apparent discrimination by the chicks.

Unless otherwise indicated, the manure was collected from the same group of lactating cows is free of urine, straw and extraneous material as possible.

EXPERIMENTAL RESULTS

I. *Effect of the temperature of drying on male hormone in cow manure.* In the original study of Riley and Hammond (11) and in later publications (4) it was reported that drying cow manure at a temperature of 80° C. caused the total destruction of the male hormone. To determine the effect of the temperature of drying of cow manure upon the male hormone content under our conditions, samples of cow manure were dried at 45°, 55°, 65°, 75°, and 85° C. in a Freas electric oven. At the lower temperatures about 48 hours were required. At the higher temperatures drying was faster, requiring only 24 hours.

The manure was then ground and fed to groups of about 20 chicks as 10 per cent of the chick starter ration. It will be seen in table 1 that as the temperature of drying manure increased there was a gradual inactivation of the male hormone present. At temperatures above 75° C. all hormone was destroyed since the comb weights were down to control levels.

From these data it appears that the slow drying of cow manure at temperatures above 45° C. for 24 hours or more inactivates increasing amounts of the male hormone present up to about 75° C. It is possible that even a

lower temperature of drying would preserve even larger amounts of the hormone, although the drying time would be increased. These data do not answer the question whether higher drying temperatures might not be employed successfully if the time of drying could be reduced to a minimum.

II. *The androgen content of manure of cattle, goats, and sheep.* The following comparisons are based upon the inclusion of 10 per cent of the manure in the chick starter ration. In each case the manure was dried at 45° C. The average weight of the combs of the control White Plymouth Rock chicks at 4 weeks of age varied from 47.8 mg. to a high of 91.0 mg., with an average of 61.3 mg. for 51 chicks. When chicks were fed manure from lactating cows, the combs were much enlarged and bright red in color. The combs of 40 chicks averaged 225.2 mg. in weight—almost a 4-fold in-

TABLE 1

Effect of drying temperature upon male hormone content of cow manure fed at 10% level
(Experiment started, Feb. 14, 1946)

Temperature of drying for 24 to 48 hours	No. of chicks	Average body weight at 28 days	Average comb weight	Comb weight 100 gm. body weight
°C.		gm.	mg.	
45	20	259.0	249.2	96.2
55	20	227.6	178.9	78.6
65	21	231.5	76.3	32.9
75	21	232.6	31.2	13.4
85	21	233.5	35.0	14.6 .

crease over the control chicks' combs. This average comb weight is quite similar to that reported by Riley and Hammond (11) for chicks fed manure from lactating cows (237.1 mg.).

In a preliminary test of mixed bull manure, the average comb weight of the chicks indicated the presence of about one-fourth as much male hormone as did the manure from lactating cows. In a further test of the manure of three Holstein-Friesian bulls varying in age from 2 to 16 years, only slight stimulation was observed.

From the data presented, it would appear that lactating goats are not similar to lactating cows in excreting large amounts of male hormone in their feces. At least, if it is excreted, it is not orally available to chicks. The sample from the pregnant, not lactating, group showed the greatest effect upon the comb. Some male hormone is excreted by the buck since the response of the comb was about double that of the controls and was greater than that shown by the bulls.

Samples from a single, non-pregnant ewe at two periods showed slight responses comparable to that of the non-pregnant goats.

From these observations, it would appear that the lactating cow eliminates a considerable amount of an androgen or androgens which are orally

available to the chick. Lactating goats and other ruminant animals both male and female appear to excrete only slight amounts by this route.

III. *Androgen in manure from lactating cows compared with methyl testosterone administered orally.* Riley and Hammond (11) fed 18 mg. of testosterone acetate per kg. of feed in one lot and 18 mg. testosterone acetate + 3.6 mg. of estrone to a second lot of chicks. The comb weights of the two groups were essentially the same. On the basis of equal numbers of the two sexes, the average comb weight of 48 chicks was 649.7 mg. Since the average comb weight of these chicks was far above the comb weight of

TABLE 2

The androgen content of dry manure of cattle, goats and sheep
(Fed as 10% of the chick ration)

Type of manure	No. of chicks	Average body weight at 28 days	Average comb weight	Comb weight / 100 gm. body wt.	Date of start of experiment
		gm.	*mg.*		
Control feed	10	305.1	56.3	18.4	Nov.
"	10	227.7	60.8	26.7	Dec.
"	11	307.4	91.0	29.6	Dec.
"	20	223.1	47.8	21.5	May
Cow, lactating	10	257.1	172.6	67.1	Oct.
"	10	296.6	229.8	77.4	Dec.
"	20	259.0	249.2	96.2	Feb.
Bull, mixed	8	230.6	119.5	51.8	Dec.
H-Bull—16 years	20	261.2	62.7	23.9	May
H-Bull— 2 years	19	219.4	59.3	26.7	May
H-Bull— 6 years	20	247.1	73.6	29.5	May
Goat, not pregnant	21	272.0	77.4	29.3	May
Goat, pregnant non-lactating	10	248.1	134.9	54.3	Oct.
Goat, lactating	21	256.9	76.5	29.2	May
Buck	19	223.0	95.2	41.1	May
Sheep, not pregnant	10	307.0	97.5	31.7	Oct.
"	20	221.3	62.9	28.4	May

chicks fed 10 per cent manure, it seemed desirable to determine the amount of synthetic male hormone administered orally equivalent to the amount of male hormone present in the manure of lactating cows.

In a preliminary experiment, groups of about 20 chicks each were fed from 0.75 mg. to 6.0 mg. of methyl testosterone[3] per kg. of feed. As will be observed in table 3, this dosage range was too low to compare with varying amounts of cow manure added to the ration, so in a second experiment the amount of hormone was increased progressively from 10.0 mg. to 40.0 mg. per kg. of feed. There was a regular increase in average comb weight of the chicks up to 30.0 mg. per kg. of feed. When this latter amount was fed dissolved in soybean oil, the comb response was slightly reduced.

[3] Kindly supplied by Dr. E. Schwenk of Schering Corp., Bloomfield, N. J.

For comparison with these data, graded amounts of cow manure were added to the chick ration to cover the same range of average comb response. It will be seen that over the range of 50 gm. to 200 gm. cow manure per kg. feed, the average comb weight compares with the effect of 10 to 30 mg. of methyl testosterone per kg. of feed. In other words, 100 gm. of dry cow manure contains the oral equivalent (in chicks) of 20 mg. of methyl testosterone or 5 kg. of cow manure contains the oral equivalent of 1 gm. of methyl testosterone.

Since 18 mg. of testosterone acetate per kg. feed produced combs weighing 649.7 mg. (11) whereas 20 mg. methyl testosterone per kg. of feed in-

TABLE 3

Comparison of comb-stimulating properties of dried cow manure and methyl testosterone administered orally

Material per kg. feed	No. of chicks	Average body weight at 28 days	Average comb weight	Comb weight 100 gm. body wt.	Date of experiment
		gm.	*mg.*		
Methyl testosterone					
0.75 mg.	21	250.7	51.0	20.3	Jan.
1.50 mg.	19	268.2	88.0	32.8	Jan.
1.50 mg. (in oil)	16	253.4	56.5	22.2	Jan.
3.00 mg.	22	264.0	98.1	37.1	Jan.
6.00 mg.	17	261.5	84.6	32.3	Jan.
10.00 mg.	20	239.3	161.4	67.4	Feb.
20.00 mg.	19	208.2	233.8	112.3	Feb.
30.00 mg.	21	234.8	348.2	148.3	Feb.
30.00 mg. (in oil)	21	234.6	321.3	136.9	Feb.
40.00 mg.	21	226.9	196.7	86.6	Feb.
Cow manure					
25.0 gm. (2.5%)	10	319.5	113.1	35.3	Dec.
50.0 gm. (5.0%)	9	298.6	155.0	51.9	Dec.
100.0 gm. (10.0%)	40	....	225.2	....	(see table 2)
200.0 gm. (20.0%)	20	264.5	386.4	146.0	Oct.–Dec.

creased combs to an average of 225.2 mg., it would appear that the methyl derivative of testosterone is less effective orally in fowls than the acetate.

IV. *Source of the androgen in cow manure.* In the fowl, part if not all of the male hormone produced by laying hens which stimulates the growth of the comb is believed to be secreted by the medullary part of the ovary (3). In the mouse, the secretion of male hormone by the ovaries is stimulated when ovarian grafts are made into the ear (5). Burrill and Greene (1) present evidence indicating the secretion of male hormone in the pregnant and lactating rat. In man, both normal male and female urine contains considerable amounts of both estrogens and androgens.

In cattle, the male hormone was observed to be excreted in small amounts in the urine of the bull (7). Little has been reported on androgn content of cow urine. The observations presented in this paper indicating the

presence of considerable amounts of androgens in the feces of lactating cattle raise the question as to the source of the male hormone and the part of the digestive tract where the androgen is passed into the contents of the tract. In addition to the ovaries, it is well established that the adrenal glands may under certain conditions secrete male hormone.

As might be expected, it has been reported that androgens are not present in dried rumen contents (4). From our knowledge of the metabolism of other hormones, the liver might be expected to play a rôle in the elimination of androgen by way of the bile from either the ovaries or adrenals, although it is possible that it might be excreted directly by the cells lining the digestive tract.

Since milking cows were not available, the entire digestive tracts of two Shorthorn type cows were obtained from a local packing house for our preliminary study. The livers and bile were also retained. The rumen contents were each sampled and dried. The contents of the omasum, small intestine and large intestine of each cow were combined and dried. The livers were dried and ground and the bile mixed with the chick feed and dried. The average weight of the combs of the chicks indicated insufficient androgen present to indicate the point in the digestive tract where the products of androgen metabolism were discharged into the digestive tract.

While the combs of the chicks fed graded amounts of liver were almost

TABLE 4

Source of androgen
(20 chicks in each lot)

Material	Amount fed	Average body wt. chicks at 28 days	Average comb weight	Comb weight / 100 gm. body wt.	Date of experiment
		gm.	*mg.*		
Cattle					
Rumen content					
Cow I	10%	234.7	72.8	31.0	April
Cow II	10%	217.7	48.0	22.0	April
Omasum content, mixed	10%	233.8	40.9	17.4	April
Small intestine, mixed	355 gm. in 10 kg.	220.5	49.3	22.3	April
Large intestine, mixed	10%	225.2	38.6	17.1	April
Dried liver	5%	275.1	63.5	23.0	April
" "	10%	295.6	74.3	25.1	April
" "	15%	298.0	75.9	25.4	April
Bile	470 ml.*	276.7	55.2	19.9	April
Goat rumen	10%	202.1	45.0	22.2	Dec.
" urine	5876 cc. dried in 10 kg. feed	233.3	52.3	22.4	April

* Since no more cattle bile could be obtained, 735 ml. of hog bile was added.

double the control comb weight, the fact that their size was not closely graded in proportion to liver dosage raises a question as to the significance of the greater comb weight.

This preliminary work clearly indicates the necessity of using lactating cows in such a study and to have evidence that the cows being used are discharging normal amounts of androgens in their manure.

DISCUSSION

The discovery of the excretion of considerable amounts of androgenic hormone in the feces of the lactating dairy cow is of considerable interest from several points of view. The use of dried cow manure in the ration of chickens and swine as a rich source of B-vitamins with nutritive value comparable to an equivalent amount of alfalfa meal raises the question of the desirability of utilizing this by-product of dairy farming as a feeding stuff rather than as a fertilizer.

In addition to its nutritive value, dried cow manure is an economical source of an orally available androgenic hormone. It has been shown in rats that the injection of androgens in suitable amounts stimulates the rate of growth (12–13). It is also recognized that this hormone favorably influences nitrogen metabolism (6). Work in this laboratory indicates that the feeding of dried cow manure at suitable levels to chicks from hatching time until sexual maturity stimulates the growth of the pullets especially, and the age of the onset of egg production was reduced.

The rôle of the male hormone in lactating dairy cows is especially intriguing. Evidence is constantly increasing indicating that both male and female sex hormones are being produced by the endocrine glands of both males and females in considerable amounts. It has been demonstrated that certain androgenic hormones stimulate the growth of the mammary glands experimentally. However, up to the present time the male hormone has not been suggested as playing a dynamic rôle in either the normal growth of the cow's udder or in the initiation or maintenance of milk secretion. This problem deserves serious consideration in the light of the observations reported.

SUMMARY

The male hormone (androgen) content of ruminant manure was assayed biologically by feeding it as part of the starter ration to groups of White Plymouth Rock chicks during a period of 28 days. The average comb weight of 40 chicks fed 10 per cent dried manure from lactating cows was 225.2 mg. in comparison to the comb weight of normal chicks of 61.3 mg.

Lactating cow manure dried at temperatures ranging from 45° C. to 85° C. by 10 degree intervals gradually declined in androgen potency. At temperatures of 75° C. and above all biological activity was lost.

The androgen content of the manure of other ruminants including goats

and sheep of both sexes and conditions was either low or absent. The manure of dairy bulls showed indications of only small androgen excretion by that route.

Chicks fed methyl testosterone at the rate of 20 mg. per kg. of feed had combs averaging 233.8 mg. This is comparable to the effect of lactating-cow manure fed at the 10 per cent level or 100 gm. per kg. of feed.

The point of entrance of the androgenic hormone into the digestive tract of the cow has not yet been determined.

REFERENCES

(1) BURRILL, M. W., AND GREENE, R. R. Androgen Production During Pregnancy and Lactation in the Rat. Anat. Rec., 83: 209. 1942.

(2) DAVID, K. Über den Einfluss der Saison auf die Empfindlichkeit des Hahnenkammes. Acta Brevia Neerl., 8: 133. 1938.

(3) DOMM, L. V. Precocious Development of Sexual Characters in the Fowl by Homeoplastic Hypophyseal Implants. II. The Female. Soc. Expt. Biol. and Med. Proc., 29: 310. 1931.

(4) HAMMOND, J. C. Dried Cow Manure and Dried Rumen Contents as a Partial Substitute for Alfalfa Leaf Meal. Poultry Sci., 23: 471. 1944.

(5) HILL, R. T. Ovaries Secrete Male Hormone. Endocrinol., 21: 495–633. 1937.

(6) KENYON, A. T., SANDIFORD, I., BRYAN, A. H., KNOWLTON, K., AND KOCH, F. C. The Effect of Testosterone Propionate on Nitrogen, Electrolyte, Water and Energy Metabolism in Eunuchoidism. Endocrinol., 23: 135. 1938.

(7) KOCH, F. C. The Male Sex Hormone. Physiol. Rev., 17: 153. 1937.

(8) McGEE, L. C. The Effect of the Injection of a Lipoid Fraction of Bull Testicle in Capons. Inst. Med. of Chicago Proc., 6: 242. 1927.

(9) McGEE, L. C., JUHN, M., AND DOMM, L. The Development of Secondary Sex Characters in Capons by Injections of Extracts of Bull Testes. Amer. Jour. Physiol., 87: 406. 1928.

(10) REINEKE, E. P., AND TURNER, C. W. Seasonal Rhythm in the Thyroid Hormone Secretion of the Chick. Poultry Sci., 24: 499. 1945.

(11) RILEY, G. M., AND HAMMOND, J. C. An Androgenic Substance in Feces from Cattle as Demonstrated by Tests on the Chick. Endocrinol., 31: 653. 1942.

(12) RUBINSTEIN, H. S., AND SOLOMON, M. L. Growth-stimulating Effect of Testosterone Propionate. Soc. Expt. Biol. and Med. Proc., 44: 442. 1940.

(13) RUBINSTEIN, H. S., AND SOLOMON, M. L. Effect on Body Growth of Small Doses of Testosterone Propionate Administered at Different Seasons. Soc. Expt. Biol. and Med. Proc., 45: 745. 1940.

(14) SCHULTZE, A. B., AND TURNER, C. W. The Determination of the Rate of Thyroxine Secretion by Certain Domestic Animals. Mo. Agr. Expt. Sta. Res. Bul. 392. 1945.

SOME OBSERVATIONS ON NERVE REGENERATION IN THE UDDER[1]

DWIGHT ESPE[2]

Iowa State College, Ames, Iowa

The theory has been generally accepted that cows "let down" their milk as a result of afferent stimuli which originate in the udder. The most common of these stimuli are nursing by the calf or milking by hand or machine. Ingelbrecht (1), working with rats, claimed that the "letting down" of the milk in the mammary gland was dependent upon an intact nerve supply. When he destroyed the nerves to the posterior mammary glands and covered the anterior glands the rats failed to lactate normally and the young soon died. But when he permitted the young rats to nurse the glands with an intact nerve supply, every gland secreted milk and the rats grew normally.

Ely and Petersen (2) consider this phenomenon due to a nerve-endocrine mechanism rather than to a nervous reflex. They suggest that the afferent impulses produced by milking cause the posterior lobe of the pituitary to release oxytocin. The oxytocin acts upon the smooth muscle fibers surrounding the alveoli in the mammary gland and forces the milk in the cells and lumina of the alveoli into the larger collecting ducts and udder cisterns. It is at this particular moment that a cow is said to have "let down" her milk.

If their theory is correct only the milk in the udder cisterns could be obtained from a cow in the absence of this nerve-endocrine stimulus. Although a cow may be so conditioned to milking that various afferent stimuli cause her to "let down" her milk, the nursing of the calf or the massaging of the udder in milking should be the primary stimuli. Ely and Petersen (2) found that cutting the inguinal nerve on three dry cows had no effect on milk secretion when the cows freshened about two months later. This procedure should have eliminated any direct effect of the nerves on the musculature of the udder, unless nerve-regeneration occurred. But cutting of the inguinal nerve does not interfere with the sensory nerve supply to the skin of the udder.

EXPERIMENTAL

In order to destroy all nerve pathways to the mammary gland, an udder transplant was made on a Holstein calf when she was about six weeks of age. The mammary glands were dissected free of the body except for one exter-

nal pudic artery which was left intact. The dissected glands were turned around so that the rear teats faced anteriorly, and the udder was then sewed back into place. After the skin lesions had healed a second incision was made on the right side of the glandular area and the external pudic artery which had previously been left intact, was ligated.

The heifer was bred at as early an age as possible. She freshened when less than 22 months of age. This may help to explain why she failed to clean normally and why she was rather slow in coming to her milk. However, she was able to provide sufficient milk for her calf and another calf which nursed her. Although the udder presented a most unusual appear-
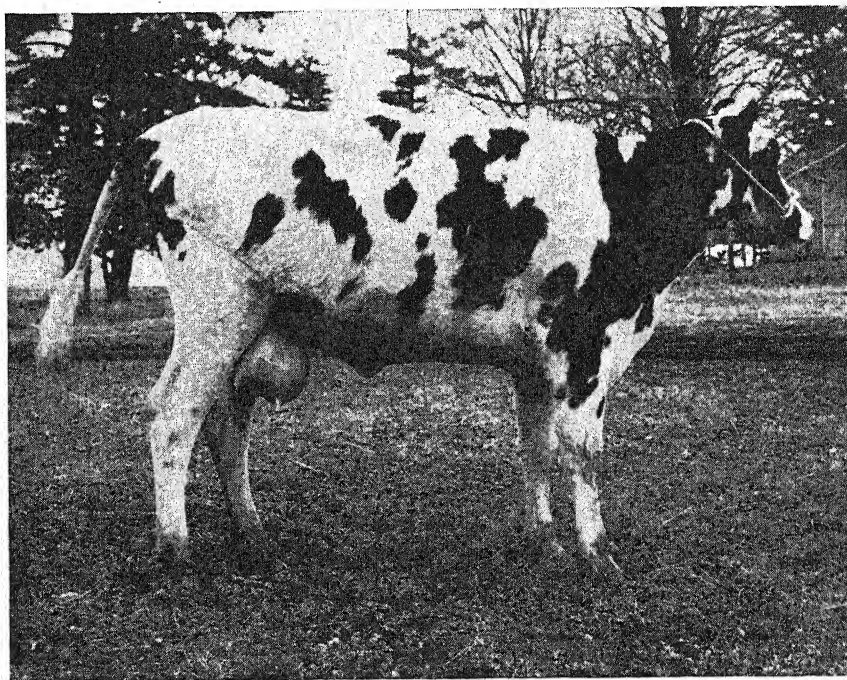
FIG. 1. Cow whose udder was reversed and all nerves and blood vessels ligated.

ance the day after parturition (fig. 1), its development during pregnancy seemed quite normal. One month after freshening the calves were weighed before and after nursing (twice a day) for three days. By this method of calculation it was found that she was producing $23\frac{1}{2}$ pounds of milk per day, in spite of the fact that the right rear gland (as now attached) gave almost no milk. Bacteriological examination showed that the gland was badly infected with mastitis. Except for this quarter the mammary glands produced an apparently normal secretion. Treatment with penicillin failed to return the diseased gland to the same production as the left rear quarter.

An electrical current from a secondary coil was used to determine the

sensitivity of the udder to external stimuli. Although the left side of the udder did not seem quite as sensitive as the skin above the incision there was no point on the left half of the udder when the electrical current did not cause the cow to jump. The entire right half of the udder was devoid of a sensory nerve supply. The only explanation the author has for this discrepancy in nerve supply between the two halves of the udder is that the regenerating nerves were destroyed on the right side of the udder when the second incision was made. However, if this explanation were valid, it seems strange that the anterior and posterior surfaces of the right half of the udder (where only the one incision was made) would be as devoid of a sensory nerve supply as the area immediately below the second incision. Every effort was made to ligate all of the mammary tissue during the two operations, yet it is conceivable that this was not done. Failure to completely sever all of the nerves seems a more probable explanation, especially since

TABLE 1

Milk obtained from transplanted udder

Quarter	Amount of milk obtained in first 4 minutes of milking	Amount of milk obtained in last 2 minutes of milking
	pounds	*pounds*
Right front	1.5*	0.7†
Right rear	Not used because of mastitis	......
Left front	3.2	0.4
Left rear	2.8	0.5

* Before any known afferent stimuli had caused the cow to ''let down'' her milk.
† After the calves have nursed for 2 minutes the glands with an afferent nerve supply (left front and left rear).

electrical stimuli when applied to the left side of the udder caused the cow to kick with her right foot.

Since this cow had never been milked by hand there should have been a minimum amount of interfering conditioned reflexes established. A milking machine with one teat cup was used for these experiments. The cow was locked in her stanchion in a box stall at the time when the calves regularly nursed. Instead of allowing the calves to nurse, the experimenter would curry the cow for a couple of minutes to secure her confidence. The milking machine was then attached as quietly as possible. After four minutes, the milking machine was removed and the calves allowed to nurse all but the gland under observation. When the calves had nursed for approximately two minutes, the milking machine was again attached to the gland under observation and milking was continued for another two minutes. The results are given in table 1.

The results presented in table 1 are too meager to offer conclusive evidence. Certainly the glands which have an intact nerve supply were emptied

much more quickly than the one "normal" gland without an afferent nerve supply.   The reader may wonder why approximately 1½ pounds of milk were secured from the right front gland before the nursing of the calves provided the essential nerve-endocrine stimulus suggested by Ely and Petersen.   Certainly this amount of milk was not already present in the gland and teat cistern.   Although no definite readings were made, the milker is positive that a goodly proportion of this milk was obtained during the last half of the four-minute milking period.   The only explanation the author can suggest is that the emptying of the udder provided a certain indirect stimulus. Although a half-inch spark to the end of the right front teat elicited no response from the cow, before the four-minute milking interval was completed she manifested a certain awareness to the action of the machine.   This was especially noticeable when the milking machine was removed.

SUMMARY

The mammary tissue of a calf was transplanted so as to sever all of the original nerve and vascular supply between the body and mammary glands. Normal development of the udder occurred during pregnancy with the exception of the right half of the udder which had no sensory nerve supply. A simple experiment on the nerve-free gland has helped to confirm the theory of Ely and Petersen in regard to the functioning of a nerve-endocrine mechanism in the "letting down" of the milk.

REFERENCES

(1) INGELBRECHT, P.   L'influence du Systeme Nerveux Central sur la Mamelle Lactante
        Chez le Rat Blanc.   Compt. Rend. Soc. de Biol., 120: 1369–1371.   1935.
(2) ELY, FORDYCE, AND PETERSEN, W. E.   Factors Involved in the Ejection of Milk.
        JOUR. DAIRY SCI., 24: 211–223.   1946.

PEPTIC–TRYPTIC DIGESTION OF RENNET–CUSTARDS IN VITRO

BERNHARD SPUR AND IRVING J. WOLMAN

Milk Research Laboratory, The Children's Hospital of Philadelphia, Philadelphia, Pa.

The present paper describes further studies in which plain and rennin-treated milks (rennet-custards) were first digested artificially by pepsin-hydrochloric acid mixtures, and then exposed to additional digestion by synthetic mixtures of pancreatin and bile.

The possibility that cow's milk can be made more easily digestible by exposure to enzymes, such as the rennet enzyme, prior to ingestion, has not been adequately explored. Both Doan and Dizikes (2) and Turner (5) have observed that milk previously exposed to tryptic (pancreatin) digestion in vitro for a short period became broken down more quickly than ordinary milk. Actual feeding of infants with enzyme-treated milks has been carried out by Blatt (1), who stated that such treated milk proved easily digestible, well tolerated and well utilized, even by premature infants.

The influence of added rennin upon curd-forming properties and peptic digestion of milk has already been discussed in a previous publication (4). This study showed that when rennet-custards were exposed to conditions of hydrochloric acid and pepsin resembling those in the normal stomach, they underwent proteolysis more rapidly than untreated milks used as controls. However, the degree of proteolysis as measured by the amount of casein made soluble in the whey filtrate was relatively small. Evidently, gastric digestion of milk is only preparatory to the major digestive activity of the small intestine.

PROCEDURE

In the experiments here reported, the artificial coagulation device (3) and the artificial digestion procedures (4) were the same as already described. The rennet-custards were prepared as follows: Lukewarm pasteurized milk was mixed with the rennet preparation undergoing test, and 100-ml. portions of the mixture poured promptly into the rubber bags in the coagulation device. These stood motionless in the water bath at a temperature of 37° C. for 5 minutes, during which time the coagulation into rennet-custards took place. Lukewarm control milks, without rennet, were added to similar bags at this time.

The apparatus was next set in motion. One ml. of a 0.6 per cent pepsin solution containing sufficient N/1 hydrochloric acid to lower the pH to 5.5 was then added to each bag. (The pH of 5.5 reproduces the approximate hydrogen ion concentration at which milk normally coagulates in the child's stomach (6).) After the control milks had coagulated, the apparatus was then agitated for one-half hour. Two ml. of the pepsin solution and sufficient

additional hydrochloric acid to bring the mixture to the desired pH were next mixed in each bag and the agitation permitted to continue for 2½ hours longer.

The rennet-custards and the control milks were handled in the same way. When rennet tablets[1] were used, the controls had the same amount of water added as did the rennet-custards, omitting the dissolved tablet; when rennet powder[1] was used, all ingredients of the powder, except the rennin itself, were added to the control milks.

The next step was digestion by pancreatin-bile solutions of varying strengths. After exposure of the milk to the pepsin-hydrochloric acid activity for 3 hours, the pH was raised to 7.5 by a strong sodium hydroxide solution, a solution of pancreatin-bile was mixed in, and the digestion continued for 2 hours more. During this period representative samples were removed at 20, 60, and 120 minutes. The digestion experiments were grouped into three series, according to the concentrations of pepsin-hydrochloric acid and pancreatin-bile used (table 1). In the first series, 3 ml. of 0.6 per cent pepsin solution, together with sufficient hydrochloric acid to produce a pH of 2.5, were mixed with 100 ml. milk. In the second series, 1 ml. of 0.6 per cent pepsin solution, together with sufficient hydrochloric acid to produce a pH of 3.5, were added. In the third series, 0.5 ml. of 0.6 per cent pepsin solution, together with sufficient hydrochloric acid to produce a pH of 5.5, were added. Thus, the concentration of pepsin in the second series was one-third, and in the third series, one-sixth of that in the first series.

The pancreatin-bile consisted of a solution of 3 gm. pancreatin powder (U.S.P. XII) plus 3 gm. bile powder (Bacto lactose peptone bile, dehydrated) in 100 ml. water. Of this solution 3 ml. were added to each experimental specimen in the first series, 1.5 ml. to each in the second series, and 0.5 ml. to each in the third series. Enough sodium hydroxide was introduced along with the pancreatin-bile mixture to keep the pH constantly at 7.5, corresponding to the hydrogen ion status of the upper part of the resting small intestine. For purposes of convenience, the three procedures have been designated as "stronger," "intermediate," and "weaker" peptic-tryptic digestion.

The amount of soluble nitrogen in the filtered whey was measured by the macro Kjeldahl method. The amount of soluble nitrogen in the blank test, *i.e.*, in the milk itself prior to any of the digestive manipulations, was sub-

[1] The rennet preparations used to make rennet-custard were: Household rennet tablets, composed of powdered rennet—2.84% and table salt—97.17% (one 0.55 gm. tablet per 500 ml. milk). Rennet powder, containing salt with added sugar and vanilla flavor, and composed of sugar 98.59%, salt—1.37% and rennin—0.04% (45 gm. of powder per 500 ml. milk). Each preparation contained sufficient rennin to coagulate 500 ml. milk in 2½ minutes at 110° F.

The tablets and powder were secured from Chr. Hansen's Laboratory, Inc., Little Falls, N. Y.

TABLE 1

	Pepsin-HCl mixture	pH	Pancreatin-bile mixture	pH
	cc.		*cc.*	
1. "Stronger" peptic-tryptic digestion	3.0	2.5	3.0	7.5
2. "Intermediate" " "	1.0	3.5	1.5	7.5
3. "Weaker" " "	0.5	5.5	0.5	7.5

tracted, and the difference taken as indicative of the rate of digestion at the end of the subsequent pancreatin-bile digestion. To measure the extent of trypsin digestion of casein, the method of the U. S. Pharmacopoeia (XII) was used. A few ml. of a specially prepared acetic acid-alcohol solution were added to the samples just before filtering. This solution precipitates that fraction of the casein which has become soluble but not yet fully broken down.

RESULTS

The mixtures of pepsin-hydrochloric acid and pancreatin-bile used in these experiments are believed to simulate the conditions prevailing in the stomach and small intestine of children at successive stages of growth. The exact amounts of the several components which would enter into the actual digestion of 100 ml. of milk are not precisely known, of course. The results of this study are to be viewed as comparative rather than absolute; they
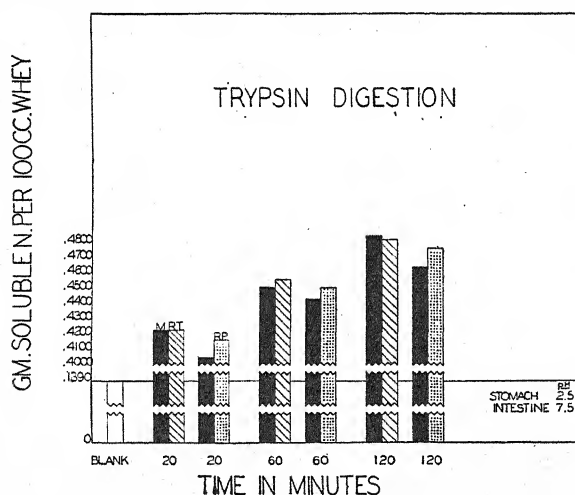
FIG. 1. Peptic-tryptic digestion of rennet-custard milks compared with pasteurized milks as controls. (Group 1 of the experiments, greatest amount of pepsin-hydrochloric acid and pancreatin-bile.) The pH in peptic digestion: 2.5; pH in tryptic digestion: 7.5; amount of pepsin used: 3 ml. 0.6% pepsin solution to 100 ml. milk; amount of trypsin (pancreatin-bile) used: 3 ml. of 3% solution of pancreatin-bile. (Average of 4-experiment series.) M = milk controls; RT = rennet-custard made with tablets; RP = rennet-custard made with powder.

serve for contrasting the breakdown rates of rennet-custards versus controls of untreated milk under a certain set of conditions, even though these conditions may not simulate exactly the conditions in the living body.

In the pepsin-hydrochloric acid digestion phase the rennet-custards produce curds which are smaller than those of the controls. These curd size differences may influence the digestion rates (4). In the later pancreatin-bile digestion, the coagula liquefy quickly and disappear. Only when the pepsin-hydrochloric acid and the pancreatin-bile preparations were both of low potency did curds of considerable size persist to the end of the process.
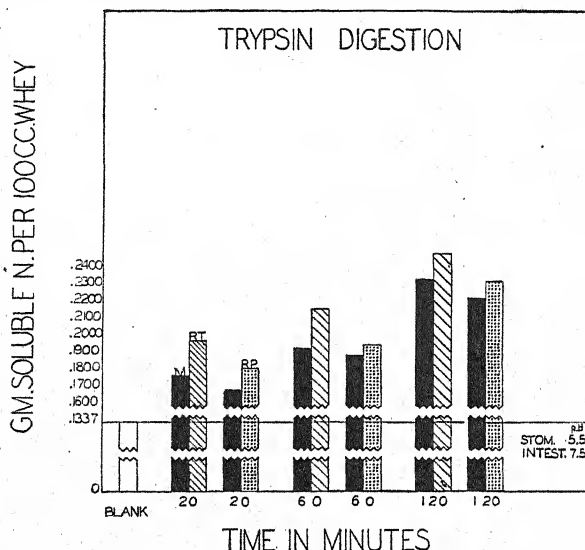
FIG. 2.   Peptic-tryptic digestion of rennet-custards compared with pasteurized market milks as controls.   (Group 3 of the experiments, smallest amount of pepsin-hydrochloric acid and pancreatin-bile.)   The pH in peptic digestion: 5.5.   Amount of pepsin $\frac{1}{6}$, and of hydrochloric acid, $\frac{1}{3}$ of amount used in group 1.   Amount of (trypsin) pancreatin-bile: $\frac{1}{3}$ of amount used in group 1.   (Average values from 6-experiment series.)   M = milk controls; RT = rennet-custard made with tablets; RP = renent-custard made with powder.

Here, as with the pepsin-hydrochloric acid digestions, the curds from rennet-custards were smaller than those from the control milks.

With the "stronger" peptic-tryptic procedure, the difference between the rennet-custards and their controls was negligible (fig. 1, table 2).

With the "weaker" peptic-tryptic procedure, the rennet-custards as made with the two preparations of enzyme lead with a slight margin over the controls (fig. 2, table 3). The pepsin-hydrochloric acid phase of the digestion presented approximately the same relative picture, but of course with less total digestion, since the hydrogen ion concentration (pH 5.5) practically excluded any peptic activity.

With the "intermediate" peptic-tryptic procedure, the rennet-custards

TABLE 2

Pepsin-trypsin digestion in vitro

Group 1

Gastric digestion: 3 ml. pepsin solution (0.6%)   pH: 2.5
Tryptic   "   : 3 ml. (trypsin) pancreatin-bile solution (3%)   pH: 7.5

Average from 4-experiment series

Milk	Digestion time	Sol. N in whey from 100 ml. of milk	Increase of sol. N in whey from 100 ml. of milk	Difference	% Casein made sol.	Diff.
	min.					
Blank test	....	0.1390	....	....	......	......
Control 1	20	0.4231	0.2841	....	73	......
#1	20	0.4231	0.2841	0.0000	73	0
Control 3	20	0.4054	0.2664	....	68	......
#3	20	0.4165	0.2775	0.0111	71	3
Control 1	60	0.4512	0.3122	....	80	......
#1	60	0.4563	0.3173	0.0051	81	1
Control 3	60	0.4435	0.3045	....	79	......
#3	60	0.4512	0.3122	0.0077	80	1
Control 1	120	0.4850	0.3460	....	89	......
#1	120	0.4824	0.3434	0.0026	88	1
Control 3	120	0.4645	0.3255	....	84	......
#3	120	0.4768	0.3378	0.0123	87	3
Genuine milk	....	0.5289	....	....	......	......

TABLE 3

Pepsin-trypsin digestion in vitro

Group 3

Gastric digestion: 0.5 ml. pepsin solution (0.6%)   pH: 5.5
Tryptic   "   : 0.5 ml. (trypsin) pancreatin-bile solution (3%)   pH: 7.5

Average from 6-experiment series

Milk	Digestion time	Sol. N in whey from 100 ml. of milk	Increase of sol. N in whey from 100 ml. of milk	Difference	% Casein made sol.	Diff.
	min.					
Blank test	....	0.1337	....	....	......	......
Control 1	20	0.1771	0.0434	....	11	......
#1	20	0.1979	0.0642	0.0208	17	6
Control 3	20	0.1685	0.0348	....	9	......
#3	20	0.1815	0.0478	0.0130	13	4
Control 1	60	0.1936	0.0599	....	16	......
#1	60	0.2160	0.0823	0.0224	22	6
Control 3	60	0.1887	0.0550	....	15	......
#3	60	0.1946	0.0609	0.0059	16	1
Control 1	120	0.2338	0.1001	....	26	......
#1	120	0.2483	0.1146	0.0145	30	4
Control 3	120	0.2226	0.0889	....	24	......
#3	120	0.2324	0.0987	0.0098	26	2
Genuine milk	....	0.5126	....	....	......	......
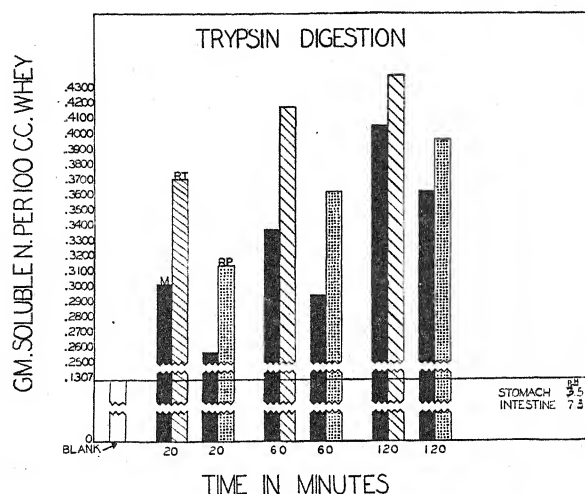
FIG. 3. Peptic-tryptic digestion of rennet-custard milks compared with pasteurized market milks as controls. (Group 2 of the experiments, intermediate conditions.) The pH in peptic digestion: 3.5. Amount of pepsin: $\frac{1}{3}$, and of hydrochloric acid: $\frac{2}{3}$ of amount used in group 1. Amount of pancreatin-bile (trypsin): $\frac{1}{2}$ of amount used in group 1. (Average values from 30-experiment series.) M = milk controls; RT = rennet-custard made with tablets; RP = rennet-custard made with powder.

showed more casein digestion than the controls; this was appreciable at 20 and 60 minutes, but less striking at 120 minutes (fig. 3, table 4).

TABLE 4

Pepsin-trypsin digestion in vitro

Group 2

Gastric digestion: 1 ml. pepsin solution (0.6%)   pH: 3.5
Tryptic   "   : 1.5 ml. (trypsin) pancreatin-bile solution (3%)   pH: 7.5

Average from 30-experiment series

Milk	Digestion time	Sol. N in whey from 100 ml. of milk	Increase of sol. N in whey from 100 ml. of milk	Difference	% Casein made sol.	Diff.
	min.					
Blank test	....	0.1307	....	....	......	......
Control 1	20	0.3030	0.1723	....	45	......
#1	20	0.3717	0.2410	0.0687	63	18
Control 3	20	0.2580	0.1273	....	33	......
#3	20	0.3145	0.1838	0.0565	48	15
Control 1	60	0.3382	0.2075	....	54	......
#1	60	0.4188	0.2881	0.0806	76	22
Control 3	60	0.2954	0.1643	....	43	......
#3	60	0.3633	0.2326	0.0683	61	18
Control 1	120	0.4061	0.2754	....	72	......
#1	120	0.4398	0.3091	0.0337	81	9
Control 3	120	0.3633	0.2326	....	61	......
#3	120	0.3971	0.2664	0.0338	70	9
Genuine milk	....	0.5117	....	....	......	......

The greater relative degree of breakdown of rennet-custards in contrast to their controls of untreated milk in some of these experiments suggests that rennin facilitates in one way or another—perhaps by opening up some peptide bindings—the peptic-hydrochloric acid activity. The greatest differences between rennet-custards and controls did not appear when the peptic and tryptic activities were strongest, as in group #1 (although this group of course showed the largest total breakdown of casein for both), or when peptic and tryptic activities were weak, as in group #3, but when the peptic and tryptic activities were moderate, as in group #2. If it is permissible to draw a clinical parallel, then this enhanced rate of digestion of rennet-custards seemingly would take place under corresponding conditions in the human body also.

SUMMARY AND CONCLUSIONS

Rennet-custards and ordinary untreated milks were subjected to experimental study in vitro to investigate the possible influence of rennin upon digestibility. The degree of digestibility was measured by the amount of soluble nitrogen which appeared in the whey after the milks had been exposed to pepsin-hydrochloric acid (peptic) digestion followed by pancreatin-bile (tryptic) digestion.

Different combinations were investigated. When strong peptic digestion was followed by strong tryptic digestion, practically no differences between the two types of milk were detectable. The same result was obtained when both peptic and tryptic activity were markedly reduced. Under intermediate digestive conditions, however, faster digestion of the rennet-custards was noticeable. For example, after the first 60 minutes of tryptic digestion, an average of 18 per cent more casein was digested in the rennet-custards than in the controls. This difference became less marked after 2 hours of digestion.

It may be concluded, therefore, that under certain experimental conditions, rennin treatment of milk speeds up digestion. It seems likely that a similar effect would take place in vivo under corresponding conditions.

REFERENCES

(1) BLATT, W. L., AND HARRIS, ELLIS. Enzylac, a Simplified Infant Food. Amer. Jour. Dis. Child., 56: 1388. 1938.

(2) DOAN, F. J., AND DIZIKES, J. L. Digestion Characteristics of Various Types of Milk Compared with Human Milk. Pa. Agr. Expt. Sta. Bul. 428. 1942.

(3) SPUR, B., AND WOLMAN, I. J. The Curd Number Test, a Method of Testing the Curdling Quality of Milk. JOUR. DAIRY SCI., 25: 409. 1942.

(4) SPUR, B., AND WOLMAN, I. J. Influence of Added Rennin Upon Curd-Forming Properties and Peptic Digestion of Milk. JOUR. DAIRY SCI., 28: 129. 1945.

(5) TURNER, A. W. Digestibility of Milk as Affected by Various Types of Treatment. Food Res., 10: 52. 1945.

(6) WOLMAN, I. J. The Gastric Phase of Milk Digestion in Childhood. Amer. Jour. Dis. Child., 71: 394. 1946.

BEST RECORDS VS. THE AVERAGE OF ALL RECORDS FOR THE EVALUATION OF A SIRE'S INHERITANCE FOR LEVEL OF PRODUCTION

R. R. GRAVES[1]

Most of the published data on which sires are proved is on the basis of averages of all records made by daughters and their dams. The sires proved in the dairy herd improvement associations, and probably in the herd tests of the breed associations, are on the basis of the averages of all the records made by the daughters and by their dams. Probably this use of the average of all records stems from the belief that a cow that has made several records has given better proof of her producing ability than the cow that has made only one record; that an average of a number of records is a better indication of the cow's real producing ability than one record; that some cows are primed for one big record and that such a record is far above her ability to produce year in and year out. The publicity that has been given to the cows making big lifetime records has probably added to the belief that the average of all records is the best medium to use for evaluating the inheritance of a sire. Most of the proof for sires is on young sires and consequently the averages usually represent a far greater number of records for the dams than for the daughters. Not infrequently the daughters may average only one record apiece while the average number for the dams may be for from 1 to 6 or more records.

In studying the proof on a large number of sires being considered for use in the Bureau of Dairy Industry's experimental breeding herds, we have been impressed with the number of sires that show up very favorably when their proof is based on the averages of several records for the dams and only one or two records for the daughters, and that show up very poorly when the proof is based on the best record made by the daughter and the best record made by the dam.

This is illustrated by the proof obtained on 16 sires loaned to cooperators by the Huntley Experiment Station. The data for these 16 sires are shown in table 1 and so arranged that a comparison is available of the average butterfat yields of the daughters, and their dams, for each sire, on the basis of both the averages of their best records and the averages of all their records. The number of daughter-dam pairs for each sire; the number of daughters that made records that were better than those of their dams, in the comparison of the best records; the number of records made by the

TABLE 1

Comparison of the best records and the average of all records of the daughter-dam pairs of 16 bulls from the Huntley Experiment Station

Sire number	Best records				All records average			
	Number of pairs	Drs. ave. fat.	Dams ave. fat.	Number better	Number of records	Drs. ave. fat	'Dams ave. fat	Number better
191	29	654	620	18	49–52	631	596	17
193	16	416	357	13	24–18	395	355	11
194	5	413	459	2	5–18	413	406	3
195	14	744	675	8	19–26	734	650	11
199	6	407	416	4	6–16	407	377	4
505	10	350	398	1	10–32	350	353	4
503	16	487	451	8	25–63	465	422	8
506	13	446	507	4	24–66	424	418	7
508	11	511	530	4	18–50	484	450	7
510	7	404	408	5	7–8	404	406	5
517	6	421	432	3	8–17	415	392	4
520	7	386	384	5	7–25	386	324	5
521	6	376	454	0	6–20	376	385	3
523	5	288	347	1	5–20	288	286	2
528	7	717	579	5	8–8	704	578	5
531	6	452	470	3	6–32	452	419	4

daughters and by the dams in the comparison of the averages of all records; and the number of daughters whose average of all their records was greater than the average for all the dams' records, is also shown in table 1. For instance, sire 191 has 29 daughter-dam pairs but in the comparison of averages of all records the daughters have 49 records and the dams have 52 records, the number of records by daughters and dams being approximately equal. Sire 503 has 16 daughter-dam pairs, and in the comparison based

TABLE 2

The per cent incr ases and the sire indexes, calculated from the best record, and also from the average of all records, as given in table 1

Sire	Per cent increase on best records	Bull index best records	Per cent increase all records	Bull index on all records
191	+ 3.5	684	+ 5.9	666
193	+16.5	475	+11.2	435
194	−10.0	367	+ 1.7	420
195	+10.2	813	+12.9	818
199	− 2.1	398	+ 7.9	437
505	−12.1	302	− 0.8	347
503	+ 8.0	523	+10.2	508
506	−12.0	385	+ 1.4	430
508	− 3.6	492	+ 7.6	518
510	− 0.9	400	− 0.5	402
517	− 2.4	410	+ 5.9	438
520	+ 0.5	388	+19.1	448
521	−17.2	298	− 2.2	367
523	−17.0	229	+ 0.7	290
528	+23.8	855	+21.8	830
531	− 3.8	434	+ 3.8	485
Average 16	− 1.22	465.7	+ 6.66	490.0

on averages of all records the daughters have a little less than two records per daughter, while the dams have almost four records per dam. In the case of sire 506 the dams average five records per dam. In the comparison for sire 531 the daughters average one record apiece and the dams average a little better than five records per dam.

The first 11 sires in table 1 are all sons of one sire; the last five are all sons of another sire.

In table 2, the data in table 1 are used to show the per cent increase in butterfat yield when the best records of daughters and dams are compared, and an equal parent sire index is calculated from the same data. The per cent increase in butterfat yield and an equal parent sire index is also shown for the data based on the average of all records made by daughters and dams. In general the data in table 2 indicate that where the number of records of the dams greatly exceeds the number of records of the daughters, in the comparison based on averages of all records, the per cent increase of the daughters over the dams and also the sire index will be much greater than the per cent increase, and the sire index, for these same animals when the comparison is based on the best records of the daughters and the dams. Note the occurrence of these increases in sires 194, 199, 505, 506, 508, 517, 520, 521, 523 and 531. There are two exceptions, namely, sire 195, where the dams have 26 records and the daughters 19 and the per cent increase is only 2.7 and the sire index is raised only 5 pounds; and 503, where the 16 dams have 63 records and the 16 daughters 25 records, and the per cent increase is 2.2 and the sire index is decreased 15 pounds. The case of 503 is most unusual.

The reverse of this trend would be expected where the daughters have a greater number of records than the dams. Only one sire, 193, illustrates this. He has 16 daughters with 24 records and the 16 dams have only 18 records, and table 2 shows that the per cent increase and the sire index based on the average of all records is smaller than the per cent increase and the sire index that is based on the comparison of the best records of the daughters and the dams.

If there is no great difference in the number of records of the daughters and dams, and if all the records are made under the same conditions there will be no great difference in the per cent increase and the sire index secured by the two methods of comparison.

This trend is probably the result of the fact that where a cow is tested year after year she is almost certain to have some bad years when her production will be below her best, and these poor years will reduce her average yield. These poor years are not necessarily due to a poor inheritance. They may be due to her having been bred too soon after calving; to an attack of mastitis; to bloat or poor hay or failure to clean properly after calving, or any one of a dozen different things that may result in her failure to produce

up to her inherent capacity.   On the other hand the two-year-old daughters
are more likely to be sound in their first lactation periods than at any other
time in their lives, and barring lack of development or the possession of an
inheritance for slow maturity, they are likely to produce at as high a level
in their first lactation period—age considered—as in any subsequent lacta-
tion.   Therefore, if the proof of a bull is based on the average production
of his daughters in their first lactation periods as compared to an average
for all the records made by their dams, that comparison is likely to be unduly
favorable to the sire.   It is a much more stringent test for the sire to base
his proof on the best records made by his daughters as compared to the best
records made by their dams.

SUMMARY AND CONCLUSIONS

Daughter-dam comparisons for 16 sires are made, one comparison on the
basis of the best records made by the daughters and the best records made
by the dams, and a second comparison on the basis of the average for all
records made by the daughters and all records made by their dams.

The per cent increase in butterfat yield and an equal parent sire index
is shown for each sire and for each of the two comparisons.

When the number of records averaged for the dams is larger than the
number of records averaged for the daughters the comparison based on aver-
ages is more likely to be unduly favorable to the sire than is the comparison
based on the best record made by the daughters and the dams.   This is due
to the fact that any cow that has a number of records is likely to have some
lactations when her production is below her best level and these low years
reduce her average yield.   The comparison is likely to be particularly favor-
able to the sire if his daughters' first records, made under favorable condi-
tions, are compared to an average of several records made by the dams.

The comparison of the best records of the daughters and the best records
of the dams is a much more rigorous test for the evaluation of the sire's
inheritance for level of production.   There will be fewer disappointments
where sires are being selected for the improvement of germ plasm for high
levels of production if they are selected on the basis of the best records
rather than on the basis of the average of all records.

THE RELATION OF INCLINATION OF RUMP TO INCLINATION OF UDDER, PRODUCTION ABILITY AND BREEDING EFFICIENCY[1]

R. E. LEIGHTON[2]

Superintendent of the United States Dairy Experiment Station at Woodward, Oklahoma[3]

AND R. R. GRAVES

Formerly Head, Division Dairy Cattle Breeding, Feeding and Management;
now President, Wood-Jon Farms, Inc., Vaparaiso, Indiana

In many cows the pin bones are lower than the hip bones, resulting in what is commonly called a "sloping rump". Perhaps the frequency of the sloping rump is decreasing somewhat. Certainly most breeders have discriminated against the sloping rump in their selection of breeding animals. Man's idea of beauty in the bovine long ago insisted that the rump should be level, in spite of the fact that most species of animals possess sloping rumps. Note the rarity of the level rump in the animals in the zoo or in the wild. Probably because the level rump was associated in man's mind with beauty, there have been developed over a period of years opinions that there are certain associated defects that are likely to accompany the undesirable sloping rump, some of them of economic importance. One such defect that received emphasis was that if the rump slopes the floor of the udder will be tilted because the floor of the udder parallels the rump, with a likelihood of the fore quarters being undeveloped. The following quotation from Van Pelt's Cow Demonstration emphasizes this idea.

> Many cows, though long in the rump, droop from the hip bones to the pin bones and are described by the expression "Drooping rumped." This conformation not only detracts from the beauty of the cow but as a rule those cows which droop at the rump, also have tilted or slanting udders a portion of which seems to have been cut away and this naturally detracts from the ability of the cow.

> On the other hand those cows which carry out straight from the hip bones to the pin bones have udders that are straight on the bottom, symmetrical and carry well forward with each quarter large and uniform in size. The fact that the length of udder can be determined by the length from hip bones to the pin bone, and the shape of the udder by the manner in which the rump is carried out, is likely due to the law of correlation of parts which enables the anatomist when he finds a bone to determine from its dimensions the dimensions of every other bone in the animal's body from which it came.

Few, if any, of these opinions have been checked by recorded observations and statistical analysis. There existed in the files of the United States

Dairy Experiment Stations, where breeding experiments have been carried on over a period of years, carefully made photographic records of the types of the animals that have constituted several generations of planned breeding that could be analyzed for the purpose of determining the relation of the angle of the rump to other body characteristics and functions. The material for this study has been confined to the records that were available at the Woodward, Oklahoma, Station.

<div align="center">PROCEDURE</div>

A series of more than 500 photographs of 155 Holstein-Friesian females in the herd of the U. S. Dairy Experiment Station at Woodward were made in connection with the inheritance investigations in progress at that station over a period of some 15 years. All females except those that die or become non-breeders are kept in the herd until they have completed at least one 365-day lactation record on official test. The size of the herd that can be maintained at the station is limited by both facilities for handling and funds. These factors have made it necessary to dispose of many of the animals by the time they are 5 years of age. Consequently the animals studied in this project were largely young cows, and they constitute an unselected cow population.

Each cow was photographed each year by a method that made all photographs comparable in scale and detail. The animals were posed on a level track, squarely in front of the camera which was mounted on a fixed post at a distance of 28 feet. No attempt was made to pose the animals so as to cover up defects; but a standardized position was used that was designed to show the actual conformation to the greatest possible extent. The same experienced photographer made and developed all of the pictures and the same camera and lens were used for the entire period. The prints were 5 by 7 inches in size and clear in detail. Legends were placed on the pictures when made. As the photographs were all made in the fall of the year it was not possible to get pictures of all of the animals when at the same stage of lactation.

In order to measure the angle of inclination or slope of the rump and udder a base line was established by drawing a line through the extreme upper attachment of the dew-claws on the front and rear legs nearest the camera. Since the cows were standing directly in front of the camera and on a level track, this proved a satisfactory line from which to measure the angles. To obtain the angle for the rump slope, a line was drawn through points at the dorsal extremity of the hip bone (tuber coxae) and the dorsal extremity of the pinbone (tuber ischii). The angle formed by the intersection of this line with the base line, or a line parallel to the base line, was measured with a protractor. To measure the udder slope, a line was drawn through the points where the front portion of the front teat and the front

portion of the rear teat join the udder. The line was extended until it intersected the base line and the angle formed was measured with a protractor. If it was evident that the pose of an animal was unnatural the measurements for that animal were discarded. If the udder was not lactating or if it appeared to be abnormal, the udder measurements were not used. The photographs were measured during the winter of 1940 by R. E. Leighton and, as a check, a number were measured again a year later. In all cases the rump slope readings were the same, but in a few cases there was as much as one degree variation in the udder slope readings.

The last animal photographs were made in 1938. In 1941 and 1942 measurements were made with a clinometer of the angle of the rump of 17 living

TABLE 1

A comparison of the degree of inclination of rump as taken from photographs and as secured from living animals

Cow number	Age of animal—years							
	1	2	3	4	5	6	7	8
	Degree of inclination							
W–212	7	11	12	15	10	......	(10)	(11)
W–223	12	11	12	12	......	......	(12)	(11)
W–235	11	11	13	......	......	(13)	(14)	......
W–245	9	11	14	.....	(13)	(12)	......	......
W–246	9	10	......	......	(10)	(10)	......	......
W–247	11	10	......	......	(9)	(8)	......	......
W–251	10	7	......	(8)	......	......	......	......
W–256	10	......	......	(11)	(10)	......	......	......
W–258	13	......	(13)	(11)	......	......	......	......
W–259	10	......	(7)	(12)	......	......	......	......
W–260	12	......	(11)	(13)	......	......	......	......
W–261	10	......	(12)	(10)	......	......	......	......
W–262	15	......	(15)	(16)	......	......	......	......
W–263	12	......	(12)	(10)	......	......	......	......
W–264	8	......	(11)	(12)	......	......	......	......
W–265	10	......	(12)	(12)	......	......	......	......
W–266	9	......	(9)	(13)	......	......	......	......

The figures enclosed in parentheses are those taken on live animals in February, 1941 and 1942.

animals in the herd. The slope of the rump for these same animals had been calculated from their pictures, though the pictures were made at an earlier age. The measurements on the living animal offer a check on the accuracy of the slopes calculated from the photographs. The results are shown in table 1, the readings made on the living animals appearing in parentheses. In view of the difference in age of the animals when photographs were taken and when the readings on the living animals were made, the results arrived at by the two methods appear quite consistent.

Only two cows had both picture measurements and clinometer readings of udder slope and these were taken 3 years apart. The photographs taken when the two cows were 3 years old showed 11° and 32° slope, respectively.

The clinometer readings made 3 years later were 23° and 32°, respectively. The increase in udder slope of the first cow was probably due to the effect of advancing age and does not indicate a lack of consistency in the slope calculated from the picture and the direct reading on the living animal.

RESULTS AND DISCUSSION

Effect of age.   To determine the effect of age on the slope of rump and the slope of the udder, averages were computed for the readings for all animals for each year, starting at 1 to 6 months for slope of rump, and at 19 to 30 months for slope of udder.   Since the animals were photographed each year as long as they were in the herd, many of the animals are repre-

TABLE 2

Effect of age upon inclination of rump and udder

Age in months	Rump		Udder	
	Animals	Inclination	Animals	Inclination
A—Average of all animals				
	number	*degrees*	*number*	*degrees*
1–6	76	10.8	......	....
7–18	124	11.4	......	....
19–30	115	12.2	50	10.1
31–42	98	13.2	82	14.1
43–54	64	13.1	59	14.7
55–66	36	14.1	33	18.3
67–78	20	13.4	18	18.0
79–90	12	13.4	12	19.0
B—Average of 20 animals with complete data from 1 to 5 years of age				
1–6	8	9.4	......	....
7–18	20	10.7	......	....
19–30	20	12.0	12	9.7
31–42	20	12.7	20	14.2
43–54	20	13.0	20	16.3
55–66	20	13.3	20	18.3

sented in each age-year group, but the younger groups are represented by larger numbers.   In order to obtain data that would bear more specifically on the effect of advance in age on the slope of rump and of udder, an additional calculation was made on 20 cows on which there were data for each age year up to 5 years, with the exception that some of these animals were not represented by pictures for study in the youngest age groups.   These two groups of data are shown in table 2, A and B.

In general the two groups show the same trend but there is some difference in magnitude of the increase in degree of slope of the rump with age. In the group shown in table 2, A, the maximum slope in the rump is attained in the age group 55–66 months, with a per cent increase in the degree of slope from the age group 1–6 months, of 30.6.   The maximum degree slope

for the 20-cow group is also reached at 55–66 months and the total per cent increase from the 1–6 months is 41.5. There is a fairly consistent increase in the slope of the rump with each age group, with the most rapid increases in the A group occurring between 7–18 months and 19–30 months, with a 7 per cent increase; and from 19–30 months to 31–42 months, with an 8.2 per cent increase. In the B group the most rapid rate of increase came earlier. From 1–6 to 7–18 months the increase in degree of slope was 13.8 per cent; from 7–18 to 19–30 months, it was 12.1 per cent; and from 19–30 to 31–42 it was 5.8 per cent. Thereafter the per cent increase in slope of rump for each age group was 2.3 per cent.

The increase in degree of udder slope was much more pronounced. For the A group the maximum degree of slope was at 79–90 months and the total increase in degree of slope at this age from the slope at 19–30 months was 88.1 per cent. For the B group the maximum degree of slope for the 20 cows was reached at 55–66 months and the total increase in degree of slope at this age was 88.6 per cent over that at 19–30 months. In both groups the greatest increase in degree of slope came from 19–30 to 31–42 months with 39.6 and 46.4 per cent, respectively. For succeeding age groups the rate of increase was very irregular, being 4.2, 24.5, –1.6, and 5.5 per cent for the animals in table 2, A; and 14.8 and 24.5 per cent for those in table 2, B.

Thus it appears that in this herd there was an increase in slope of rump up to 5 years of age, with the most rapid increases in slope falling between 1 and 3 years of age. There was an increase in slope of udder up to 7 years, with the most rapid increases taking place between 2 and 3 years of age. The maximum degree of slope of the udder attained at mature age was approximately 40 per cent greater than the maximum degree of slope of the rump at mature age.

The above observations have applied to the averages secured from all animals in each age group. There were many individual animals that varied from this trend. It was observed that in some cases there appeared to be an improvement in the levelness of the rump, even when the measurements showed an actual increase in the degree of slope. The apparent improvement was probably due to a raised tail setting.

The relation of rump inclination to udder inclination. It has been pointed out that there is a common belief that the sloping rump is associated with the tilted udder. To determine whether there was any relationship between sloping rumps and tilted udders in the animals in the Woodward Station herd, correlation coefficients were run on 87 pairs, using the readings for animals in the 31–42 months age-group. This group has the largest population for udder inclination and the third largest population for rump inclination (see table 2, A). The coefficient secured was +0.021 ± 0.11, indicating that there is no relationship between the sloping

rump in the animals in this study and the tilted udder.   Correlation coefficients were run on these same 87 pairs to determine if there was any relation between the sloping rump and producing ability; or between the tilted udder and producing ability.   The coefficient for sloping rump and producing ability was − 0.013 ± 0.107, indicating that the relationship was not significant.   The coefficient for tilted udder and producing ability was − 0.196 ± 0.103, indicating that this relationship was not significant.

Thus it appears that in this group one is as likely to find a tilted udder on a cow with a level rump as on a cow with a sloping rump; or a cow with a sloping rump is no more likely to possess a tilted udder than is the cow with a level rump.   Furthermore, producing ability, at least within the levels of production possessed by the cows in this study, is not effected by the degree of slope of either the rump or the udder.

Some observers believe that cows that have sloping rumps are more likely to have difficulty delivering calves than are cows with level rumps.   Our records were not complete enough to permit a study of the relationship of degree of slope of rump to difficult calving, but we did run a correlation coefficient on the relationship of degree of slope of the rump to breeding efficiency, as measured by the number of services per conception.   It is recognized that there are many factors that may cause a lowered breeding efficiency and it was hardly expected that the correlation coefficient for sloping rump and breeding efficiency would be significant.   The coefficient was + 0.220 ± 0.088.   While not high enough to be considered significant, it indicated a more pronounced trend than the other three coefficients that have been discussed.

The inheritance of the degree of rump and udder inclination.   It appears from the data presented that neither the sloping rump nor the tilted udder has any influence on the producing ability of an animal.   Also the levelness of the rump, or the degree of its slope, is not associated with either the levelness or the degree of tilt of the udder.   Nevertheless animals with level rumps and/or level udders have much greater sales value than do animals that have sloping rumps and/or tilted udders, other points being equal. This will be true even though it is fully understood that these features do not have a bearing on producing ability, because of the fact that the level rump and the level udder are commonly associated with the correct type or beauty of an animal.

So far as is known there are no environmental conditions that will cause a level rump to become sloping.   Photographic records in the Bureau of Dairy Industry, portraying the development of experimental breeding animals from calfhood to maturity, do show, however, that many animals undergo startling changes in the conformation of the rump.   Some that are level become sloping and some that are sloping become level.   The reasons for these changes are not known.

There are a number of known causes for changes in the shape of the udder. Injury or infection in one or more quarters may cause atrophy of the tissues that may result in an unbalanced udder. Investigations have shown that the average cow secretes in the rear quarters approximately 60 per cent of the total amount of milk produced. The greater weight of the milk in the rear quarters may result in a more pronounced sagging in these quarters. Then there is the not inconsiderable weight of the udder that may cause a relaxation of the supporting tissues. The average weight of 17 empty udders from cows at the Beltsville station that had been in milk 3 months or less at the time of slaughter was 72.98 pounds.

An investigation of the "Arrangement of the tissues by which the cow's udder is suspended,"[4] by W. W. Swett, P. C. Underwood, C. A. Matthews, and R. R. Graves, showed that probably the main supporting tissue of the udder was the fan-shaped septum between the two halves of the udder that attaches directly to the abdominal wall. If this main supporting tissue, or the lesser supporting tissues, becomes lax and stretches, because of hereditary tendencies, old age, or ill health, the shape and the balance of the udder may change quickly. The illustrations of these tissues in the publication cited show more clearly than can words how this may occur.

There was a greater probability of error in measuring the inclination of the udder than in measuring the inclination of the rump. This was because of the short distance between the base points—the base of the front and rear teats—from which the angle of the inclination of the udder was projected; the difficulty in accurately locating the teat base with some types of teats, especially those that are inclined to balloon or funnel out; and the differences that existed in the stage of lactation of different animals when photographed. The greater possibility of environmental factors influencing the shape of the udder and the greater probability of error in obtaining the correct angle of the inclination of the udder result in greater variations and more indefinite trends in the following study of the inheritance of the udder inclination than is the case in the study of rump inclination.

The data obtained in this study have been analyzed for indications of the part that inheritance plays in determining the extent of the inclination of the rump and the udder.

Table 3 shows the average inclination of the rump and of the udder of the daughters and of their dams, of the five sires used in the station herd. The data for the outbred and inbred daughters are shown separately. The program of taking photographs of each animal in the breeding herd each year had to be discontinued a few years ago, so that data are not available for all the daughter-dam pairs for sires 4 and 5. In reading the per cent increase or decrease in inclination in this table it should be kept in mind that a plus per cent indicates that the degree of slope of the daughters has

[4] Journal of Agricultural Research, Vol. 65, No. 1.

increased over that of their dams, and the minus per cent indicates the degree
of slope of the daughters has decreased or the rump or udder of the daugh-
ters has become more level as compared to that of the dams.

The inbred daughters of sire 1 and the outbred daughters of sire 2 had
the most sloping rumped daughters, each with an average of 13.5°; but the
degree of slope of sire 1's inbred daughters was 10.6 per cent greater than
the slope of their dams, while the degree of slope of sire 2's daughters was
only 3.9 per cent greater than that of their dams. Sire 5's daughters had
the least slope of any sire's daughters and they had 27.6 per cent less slope
than did their dams. This was the greatest change from the average incli-
nation of the rump of the dams shown by any group of daughters. Sires 3
and 4 were mated to dams that had the highest average rump inclination
and sire 3's outbred daughters average 6.6 per cent less inclination than
their dams and sire 4's daughters averaged 12.6 per cent less than their dams.

The per cent changes in udder inclination were much greater than those
for the rump. Perhaps this is because the changes in udder inclination were
due to some extent to environmental factors.

The greatest increase in udder inclination was in the daughters of sire 1.
The dams of his outbred daughters had more level udders than did any other
group of dams. The inbred daughters of sire 1 had udders with a greater
inclination than did his outbred daughters, but the per cent increase in in-
clination was less.

Coefficients of correlation indicated that there was no relationship be-
tween the sloping rump and the tilted udder. The results shown in table 3
show why a correlation coefficient for these characters would be without sig-
nificance. Sire 2's outbred daughters ranked with sire 1's inbred daughters
in possessing the most sloping rumps but sire 2's daughters also had the most
level udders. He increased the degree of inclination of rump of his daugh-
ters over that of their dams by 3.9 per cent and he decreased the udder
inclination of his daughters 23.8 per cent. On the other hand, sires 3 and 4
decreased the rump inclination of their daughters and greatly increased
their udder inclination.

From the data in table 3 equal parent indexes have been calculated for
each sire for inclination of rump and for inclination of udder, on the as-
sumption that the average inclination of the rump or udder of the daughters
represents the average of the degree for that character transmitted by the
parents. These indexes for sires 1, 2, and 3 are used in table 4, along with
the measurements of certain cows that they were mated to, to show the ex-
pected inheritance for inclination of rump and udder as compared to that
actually secured in full sisters. Thus the index for inclination of rump
calculated from the outbred daughters of sire 1 and their dams is 12.8°, and
the index for inclination of udder is 27.2°. Sire 1 was mated to cow W–14,
whose inclination of rump was 9.0°, and of udder 1.0°. The average of sire

TABLE 3

Comparison of degree of rump and of udder inclinations of daughters–dams of five sires

Sires	Number pairs	Rump inclination			Udder inclination		
		Daughters	Dams	Increase or decrease of daughters	Daughters	Dams	Increase or decrease
		degrees	degrees	per cent	degrees	degrees	per cent
No. 1, outbred	5	11.8	10.8	+ 9.2	17.6	8.0	+120.0
No. 1, inbred 75%*	9	13.5	12.2	+10.6	22.0	14.3	+ 53.8
No. 1, inbred 87.5%†	1	16.0	17.0	− 5.9	20.0	22.0	− 9.0
No. 2, outbred	20	13.5	13.0	+ 3.9	9.3	12.2	− 23.8
No. 2, inbred 62.5%‡	1	12.0	10.5	+14.3	12.0	20.0	− 40.0
No. 3, outbred	18	12.7	13.6	− 6.6	16.1	11.0	+ 46.3
No. 3, inbred 75%	3	13.0	14.6	−10.9	13.3	14.0	− 5.0
No. 4, outbred	4	11.8	13.5	−12.6	19.7	13.0	+ 51.5
No. 5, outbred	8	7.6	10.5	−27.6	11.0	§	....

* 75% inbreds, result of mating daughter to sire.
† 87.5% inbreds, result of mating 75% inbred daughter to sire—3 crosses of sire.
‡ 62.5% inbreds, result of mating granddaughter to sire.
§ Pictures on dams of daughters as 3-year-olds not available for measuring inclination of udder.

TABLE 4

Comparison of degree of rump and of udder inclinations of full sisters with parent index

Sire and dam	Rump inclination (degrees)		Udder inclination (degrees)	
	Parents	Daughters	Parents	Daughters
Sire No. 1	12.8		27.2	
Dam W-14	9.0		1.0	
Equal parent index	10.9	W-36, 14; W-41, 8	14.1	W-36, 10; W-41, 19
Sire No. 2	14.0		6.4	
Dam W-14	9.0		1.0	
Equal parent index	11.5	W-77, 13; W-63, 12; W-88, 8	3.7	W-77, 8; W-63, 18; W-88, 11
Sire No. 1	12.8		27.2	
Dam W-16	10.0		10.0	
Equal parent index	11.4	W-39, 12; W-47, 8	18.6	W-39, 23; W-47, 25
Sire No. 2	14.0		6.4	
Dam W-18	14.0		11.0	
Equal parent index	14.0	W-68, 11; W-81, 17	8.7	W-68, 0; W-81, 12
Sire No. 2	14.0		6.4	
Dam W-23	9.0		13.0	
Equal parent index	11.5	W-69, 13; W-79, 11	9.7	W-69, 8; W-79, 0
Sire No. 1 (inbred)	14.8		29.7	
Dam W-25	15.0		11.0	
Equal parent index	14.9	W-42, 16; W-33, 10	20.3	W-42, 22; W-33, 14
Sire No. 2	14.0		6.4	
Dam W-25	15.0		11.0	
Equal parent index	14.5	W-80, 13; W-86, 11; W-98, 16	8.7	W-80, 12; W-86, 2; W-98, 15
Sire No. 2	14.0		6.0	
Dam W-38	17.0		11.0	
Equal parent index	15.5	W-93, 13; W-54, 17	8.7	W-93, 13; W-54, 22
Sire No. 2	14.0		6.4	
Dam W-44	18.0		18.0	
Equal parent index	16.0	W-94, 14; W-223, 12	12.2	W-94, 2; W-223, 0
Sire No. 3	11.8		21.0	
Dam W-55	12.0		9.0	
Equal parent index	11.9	W-226, 9; W-95, 14	15.0	W-226, 17; W-95, 12
Sire No. 3	11.8		21.0	
Dam W-77	13.0		8.0	
Equal parent index	12.4	W-213, 14; W-99, 14; W-233, 10	14.5	W-213, 19; W-99, 12; W-233, 19
Sire No. 3	11.8		21.0	
Dam W-79	11.0		0.0	
Equal parent index	11.4	W-219, 10; W-203, 11	10.5	W-219, 10; W-203, 14

1's index for inclination of rump, and of cow W–14's actual inclination of rump, was 10.9°, the average inheritance for inclination of rump from these two parents. Inclination of the rump of the two full sisters resulting from the mating of these two parents, W–36 and W–41, is 14° and 8°, respectively. Likewise, the average index for these two parents for inclination of udder is 14.1 and the inclination for udder of W–36 and W–41 is 10° and 19°, respectively.

Such comparisons can only be of value in showing trends for the reasons that both parents were undoubtedly heterozygous for the factors determining degree of slope and consequently no one index figure can represent the range that they will transmit at different matings; and also because the

TABLE 5

The ''expected'' inheritance from the parents and the ''actual'' phenotypic inheritance received, as shown by the average inclinations of full sisters

Parents	Inclination of rump		Inclination of the udder	
	Expected index	Actual average of daughters	Expected index	Actual average of daughters
	degrees	*degrees*	*degrees*	*degrees*
1 × 14	10.9	11.0	14.1	14.5
2 × 14	11.5	11.0	3.7	12.4
1 × 16	11.4	10.0	18.6	24.0
2 × 18	14.0	14.0	8.7	6.0
2 × 23	11.5	12.0	9.7	4.0
1 × 25	14.9	13.0	20.3	18.0
2 × 25	14.5	13.3	8.7	9.7
2 × 38	15.5	15.0	8.7	17.5
2 × 44	16.0	13.0	12.2	1.0
3 × 55	11.9	11.5	15.0	14.5
3 × 77	12.4	12.7	14.5	16.7
3 × 79	11.4	10.5	10.5	12.0
Average	12.9	12.2	12.0	12.5

index as calculated for the sire is a genotypic expression of his inheritance for these characters, while the measurement of the dam and of each daughter for degree of inclination is a phenotypic expression. Nevertheless the data in this table do show trends that appear to indicate that the inclination of the rump, and to a less extent the udder, is controlled largely by inheritance.

The data in table 4 give some idea of the variability of full sisters for these two variables. The daughters of W–14 and W–25 are especially interesting because each of these dams has daughters by sires 1 and 2. The daughters of cow W–25 by sire 1 are inbred and the index for sire 1 in this case is calculated from the averages of his 9 inbred daughters and their dams. Note the variability in the udder inclination of the three daughters of sire 2 and W–14, though the index of the parents was only 3.7. Also note the low range of variability of udder inclination in the two daughters of sire 2 and cow W–44, though the parent index was 12.2.

In order to show the trend more clearly the data in table 4 are condensed and given in table 5 as the "expected" and the "actual" inclinations occurring in the daughters.

The expected inheritance for inclination of rump for the 12 parental combinations shown in table 5 varies from 10.9 to 16.0°. As is to be expected in the matings of heterozygous parents, there is considerable variation among the full sisters resulting from each parental combination. When, however, the readings for the full sisters are averaged, the results fit quite closely to the expected inheritance for inclination of rump. Thus the "expected" for the first 2 parents is 10.9° and the average of their 2 daughters is 11.0°; and for each of the other parental combinations the results are: 11.5 to 11.0, 11.4 to 10.0, 14.0 to 14.0, 11.5 to 12.0, 14.9 to 13.0, 14.5 to 13.3, 15.5 to 15.0, 16.0 to 13.0, 19.9 to 11.5, 12.4 to 12.7, and 11.4 to 10.5°. The average for the expected for the 12 parental combinations is 12.9° and the average of the full sisters of the different parental combinations is 12.2°. Thus it appears that inclination of rump is clearly an inherited character; and of a multiple factor type.

The "expected" inheritance for inclination of udder covers a much wider range than the "expected" inheritance for inclination of rump, ranging from 3.7 to 20.3°. There is also a greater departure from the "expected" in both the individual daughter and in the average of the full sisters. This is to be expected in view of the greater part that environment may play, as well as the possibility of greater error in measurement. Though there is not as close a fit of the "actual" to the "expected" as in rump inclination, the trends of the "actual" and "expected" are in sufficient agreement to indicate that inheritance plays a major part in determining the extent of the inclination of the udder.

The comparison of the "expected" to the "actual" as expressed by the average of the degree of udder inclination of the full sisters from each parental combination, reading from top to bottom, is as follows (table 5): 14.1 to 14.5, 3.7 to 12.4, 18.6 to 24.0, 8.7 to 6.0, 9.7 to 4.0, 20.3 to 18.0, 8.7 to 9.7, 8.7 to 17.5, 12.2 to 1.0, 15.0 to 14.5, 14.5 to 16.7, and 10.5 to 12.0°. The average for the "expected" is 12.0° and for the actual 12.5°. The daughters of sire 2 and W–14 greatly exceed the parental index; also the daughters of sire 2 and W–38. The 2 daughters of sire 2 and W–44 and the daughters of sire 2 and W–23 have much more level udders than was to be expected from the parental index. On the whole, however, where the parental index is high the average of the daughters is high, and where the parental index is low the average of the daughters is low. It appears that the degree of slope of the udder is also a blended inheritance but that the inheritance picture is far more likely to be distorted by environmental factors than is the degree of slope of the rump.

TABLE 6

Variation in rump and udder slope and milk and butterfat production by groups according to sire of the groups

Group	Average rump slope, age 3 years			Average udder slope, age 3 years			Average production, mature equivalent				
	Animals	Degrees	Coefficient of variation	Animals	Degrees	Coefficient of variation	Animals	Milk	Coefficient of variation	Butter fat	Coefficient of variation
	number						*number*	*pounds*		*pounds*	
Daughters of sire 1	14	11.3	28.3	14	12.8	50.0	14	18,735	11.9	595	12.0
75 per cent inbred daughters of sire 1	9	13.6	19.2	9	22.0	25.4	9	16,321	22.0	509	23.0
Daughters of sire 2	20	13.9	25.2	20	11.0	55.0	20	20,819	12.7	685	11.0
Daughters of sire 3	22	12.8	18.7	22	15.0	36.0	22	17,458	20.2	624	19.9
75 per cent inbred daughters of sire 3	4	12.0	6.7	4	14.0	18.0	3	16,418	5.3	607	12.2
Daughters of sire 4	6	13.0	25.3	6	18.0	45.0	6	18,896	9.4	678	12.1
Daughters of sire 5	6	8.6	39.5	6	11.0	17.3	3	17,718	7.2	706	6.6

Most of the records were made between 2 and 3 years of age.

VARIABILITY

Coefficients of variability were calculated for the daughters of the five sires, on which data were secured on the inclination of the rump and the udder, for milk yield, butterfat yield, inclination of the rump, and inclination of the floor of the udder. Coefficients for these characters were calculated separately for the inbred daughters of sires 1 and 3. These coefficients are shown in table 6 along with average inclination of the rump and of the floor of the udder, and the average milk yields and the average butterfat yields of the daughters of the five sires.

The outbred daughters of sire 1 were less sloping on the average than his inbred daughters, but the variation in degree of slope was far greater in the outbred daughters. His inbred daughters also had less variability in slope of the udder floor. In this case inbreeding brought about a more uniformly sloping rump and a more uniformly sloping udder floor, but when it came to milk yield and butterfat yield the inbred daughters had lower yields, and the variability of the yields was far greater than that of the outbred daughters. The greater variability in milk and butterfat yields in the inbred daughters is in accordance with findings in an experiment with inbred dairy cattle at the Beltsville Station of the Bureau of Dairy Industry, the results of which will be published in a technical bulletin of the United States Department of Agriculture. On the other hand, the variability was lower in the inbred daughters of sire 3 than in his outbred daughters for all four variables. However, there were milk and butterfat yields on only three daughters.

The daughters of sire 5 had the lowest average slope of rump of the daughters of the five sires, but the coefficient of variation was the greatest. The daughters of sire 5 and the daughters of sire 2 had the lowest average inclination for udder floor, but the daughters of sire 5 had the lowest coefficient of variation and the daughters of sire 2 had the highest coefficient of variability for this character. Perhaps these are additional indications that the slope of rump and slope of udder floor are inherited independently of each other.

In view of the history of the daughters of sire 3 at the Woodward Station, it is surprising that his daughters do not show a greater slope of the udder floor, and an even greater coefficient of variability for this character and for milk yield and butterfat yield. The daughters of this sire suffered rather uniformly at calving time from a severe inflammation of the udder and an edematous condition that persisted long after calving. It was thought that the severity of this condition resulted in some breaking down of the udder and interference with the expression of their normal inherent producing ability.

SUMMARY AND CONCLUSIONS

1. Man's ideas of beauty in the bovine long ago insisted that the rump should be level, though most species of animals have sloping rumps. Breeders for many years have selected for level rumps. But the inheritance for the sloping rump still persists, and is continually occurring in all breeds of dairy cattle, though with decreasing frequency. Along with the selection of breeding animals for level rumps, there have been developed over a period of years opinions that there are certain associated defects that are likely to be inherited along with the sloping rump. Of these opinions the most common and the most important were: (a) that the floor of the udder would parallel the rump—that with a sloping rump there would be a tilted udder; (b) the tilted or sloping udder would be deficient in the front quarters and would, therefore, have less capacity; (c) that difficult calving and lower breeding efficiency occurs in cows with sloping rumps.

2. In the experimental breeding herds of the Bureau of Dairy Industry animals were photographed once a year under carefully controlled conditions as to posing on a level track with camera on a fixed post at a uniform distance from animal and at uniform height from ground, and with the same camera used for all the pictures. It was found that these pictures could be satisfactorily used in obtaining the degree of slope of the rump; they can also be used for determining the degree of slope of the udder, though with somewhat less accuracy than that of the rump. The degree of accuracy of the slope obtained from the photographs was checked with a clinometer on the living animal and found to be satisfactory.

3. The material for this paper was obtained from a series of more than 500 photographs of 155 females in the experimental breeding herd of Holstein-Friesian cattle at the United States Experiment Station at Woodward, Okla.

4. The animals with the straightest rumps were in the age group of 1 to 6 months. From that age the slope of the rump became progressively greater up to the age of 5 years. The most rapid change in the slope of rump came at the younger ages. In one group the per cent increase in degree of inclination of the rump from the age group of 1–6 months to the age group of 7–18 months was 13.8; from 7–18 months to 19–30 months the degree of inclination was 12.1 per cent greater, and from 19–30 to 31–42 months, the increase was 5.8 per cent.

5. The increase in degree of udder slope with advance in age was more pronounced than the change in rump slope. The maximum slope of udder was at 79–90 months of age, and the per cent increase in the degree of slope at this age was 88.1 per cent greater than the degree of slope in the 19–30 month age group. As with the rump, the greatest increase in the slope of

the udder came in the younger age groups with decreasing increments with advance in age.

6. The correlation coefficient for the relationship of the degree of inclination of the rump to the degree of inclination of the udder for 87 animals was $+0.021 \pm 0.11$; the correlation coefficient for relation of degree of sloping rump to producing ability was -0.013 ± 0.107; the correlation coefficient for relation of degree of inclination of the udder to producing ability was -0.196 ± 0.103. None of these coefficients was significant.

7. Herd records were not sufficiently complete to determine the relationship of the sloping rump to difficult calving. A correlation coefficient on the relation of the degree of inclination of the rump to the number of services per conception was $+0.220 \pm 0.088$. While hardly significant, this coefficient does show a more pronounced trend than the other three coefficients.

8. Study of the data on the daughters of the different sires used in the experiment indicates that the sloping rump and the tilted udder are inherited characters, probably of a multiple factor or blending type, and that the slope of the rump and the tilt of the udder are inherited independently of each other. The average of the actual slope of either the rump or the udder of several full sisters fits quite closely the expected slope calculated from the equal parent index of all the daughter-dam pairs for the sire and the actual slope of the dam.

9. Variability was decreased in the slope of rump and in the slope of the udders of the inbred daughters of one sire while the variability in milk and butterfat yields was increased. In a second sire the inbred daughters had a lower variability than the outbred daughters in slope of rump, slope of udder, milk yield, and butterfat yield, though the number of inbred daughters was rather small. The degree of variability was not associated with the degree of the slope of either the rump or the udder.

CAN GOOD PRODUCING COWS BE FED IN SUCH MANNER AS TO MAINTAIN THEIR WEIGHT?

R. R. GRAVES[1]

Students of dairy cattle nutrition often say that dairy cows should be so fed that they will not lose weight; that if a good producing cow is losing weight she is not receiving enough nutrients. If she is producing well and is not gaining or losing in weight she is correctly fed. In other words the weight of the cow during the lactation period is one of the best guides to good feeding.

A few years ago we conducted feeding experiments at field stations to determine the relative production of cows when on limited grain rations and on rations of roughage alone and when they were receiving what we termed a full grain ration, that is when they were fed grain at the rate of 1 pound to each 3 pounds of milk produced. It was noted that the cows on roughage alone lost weight quite rapidly early in the lactation period and then gained slowly during the remainder of the period. This indicated, apparently, that the cows were unable to eat enough of the bulky roughage ration to meet their requirements for production and maintenance in the early months of lactation. This is probably the reason why these cows did not approach more closely, when on a good quality of alfalfa hay, their level of production when receiving the full grain ration, rather than because of any deficiencies in the hay.

In one experiment 11 cows were fed throughout a 12-month lactation period on a ration consisting entirely of alfalfa hay, and 8 of these cows were fed through a second successive lactation period on alfalfa hay alone.[2] In the first lactation period these 11 cows lost in the first month an average of 107 pounds, in the second month 43 pounds and in the third month 7 pounds; in the fourth month they gained 4 pounds, in the fifth month they lost 8 pounds, and thereafter they gained in weight each month. Their total average loss, from their "after-calving" weight, during 4 of the first 5 months of the lactation period was 165 pounds, and their total average gains during the remaining 8 months was 122 pounds; therefore, in the twelfth month they had failed to regain their after-calving weight by 43 pounds.

The 8 cows that completed a second successive lactation period on alfalfa hay alone lost weight only in the first 2 months of lactation—an average of 91 pounds for the 8, and during the remainder of the lactation period gained

123 pounds, so that they weighed an average of 33 pounds more in the twelfth month than their after-calving weight.

In an experiment at the Utah Station[3] 12 cows were fed through three successive 10-month lactation periods, in one of which they were fed only alfalfa hay and pasturage in season; in a second alfalfa hay, and ground barley fed at the rate of 1 pound to each 5.7 pounds of milk produced, plus pasturage in season; and in a third alfalfa hay and corn silage and pasturage in season. The weights of these 12 cows followed a very similar pattern during the three lactation periods. In the first they lost 104 pounds the first month and 17 pounds the second month—a total average loss of 121 pounds in 60 days. They gained it all back in the next 8 months and were 10 pounds over the after-calving weight in the tenth month. In the second lactation period, the one in which they received ground barley in addition to alfalfa hay, they lost an average of 105 pounds the first month, 14 pounds the second, and 4 pounds the third month, a total shrinkage of 123 pounds in the first 3 months of lactation. In the next 7 months their total average gain was 104 pounds so that in the tenth month of lactation they lacked 19 pounds of regaining their after-calving weight. In the third lactation period, when they received alfalfa hay and corn silage, the average loss of the 12 cows was 105 pounds the first month and 15 pounds the second month —a total loss of 120 pounds. Starting with the third month they made some gain each month, making a total average gain at the end of the tenth month of 154 pounds and putting them 34 pounds over their after-calving weight. Such uniformity of losses and gains for 12 cows over three lactation periods is quite remarkable.

So far as we could determine these severe losses in weight caused no physical injury to these cows, but it was no doubt associated with a lower level of production than would have been the case had they been fed in such manner as to enable them to maintain a more uniform weight. But can good dairy cows be fed so that there will be little change in their weights during the lactation period? A study was made of the changes in weight through a 12-month lactation period of 20 2-year-old Jerseys on Register of Merit test at the Lewisburg, Tenn., Field Station, of 20 2-year-old Holstein heifers on Advanced Registry test at the Huntley, Mont., Field Station, of 9 2-year-old Holsteins and 9 mature Holsteins on Advanced Registry test at the Beltsville Station, and of 9 2-year-old Jerseys and 9 mature Jerseys on Register of Merit test at the same station.

At the Lewisburg Station cows on Register of Merit test are fed legume hay *ad lib,* and a grain mixture fed at the rate of 1 pound to each 3 pounds of milk produced. They are also on pasture during the pasture season. At the Huntley Station cows are fed alfalfa hay *ad lib,* corn silage, and a grain mixture at the rate of 1 to 3. They are not pastured. At the Beltsville

[3] U.S.D.A. Tech. Bul. 724, April, 1940.

Station the method of feeding is somewhat different. The requirements of the cows for maintenance and production are calculated every 10 days. After allowing for the alfalfa hay and corn silage consumed, enough of a grain mixture is fed to provide total digestible nutrients 10 per cent in excess of the requirements. Some heavy-producing cows will not consume enough feed early in their lactation periods to provide the 10 per cent over requirements, but they were fed as closely to that level as their consumption permits. This no doubt results in somewhat more liberal feeding than is the case at the Lewisburg and Huntley Stations.

THE JERSEYS

The Beltsville 2-year-old Jerseys had an average weight of 868 pounds 13 days before calving, the individual weights ranging from 749 to 1,010 pounds. Their average weight 11 days after calving was 803 pounds, ranging from 695 to 900 pounds. The loss in weight from the before-calving to the after-calving was approximately 8 per cent of the latter weight. In the tenth month of lactation all but one of the 9 2-year-olds had reached a weight that exceeded her pre-calving weight. The one exception had the heaviest before-calving weight.

In the twelfth month of lactation they had reached an average weight of 951 pounds, a gain of 148 pounds over the after-calving weight, or 18.4 per cent. The lowest gain was 109 pounds and the highest gain was 214 pounds.

These 9 2-year-old Jerseys were evidently able to get on full feed quickly for four of them had no monthly weight that was below their after-calving weights and two more had monthly weights that were only 2 pounds and 7 pounds below their respective after-calving weights. The remaining 2 reached low weights that were 19 pounds and 42 pounds below their respective after-calving weights. They were one pound below the average after-calving weight in the first month of lactation but thereafter they made a gain each month.

Three of these heifers did not conceive during the lactation period, but they did not affect the result since their average gain from the after-calving weight to the twelfth month weight was 140 pounds, while the gain for the other 6 heifers was 153 pounds and they carried their calves an average of 188 days during the lactation period.

Feeding nutrients at the rate of 10 per cent in excess of requirements enabled them to gain consistently through the lactation period, to exceed their after-calving weights by an average of 148 pounds and to produce at the average rate of 8,994 pounds of milk, 479 pounds butterfat, starting at the age of 2 years, 1 month.

The 20 2-year-old Jersey heifers at the Lewisburg, Tenn., Station had an average before-calving weight of 769 pounds—99 pounds lighter than the before-calving weights of the 9 2-year-old Jersey heifers at Beltsville.

They ranged from 630 to 890 pounds. Their average after-calving weight was 729 pounds, with a range from 560 to 830 pounds. Thus the Lewisburg 2-year-olds were 12.8 per cent lighter than the Beltsville Jerseys before calving, and 10 per cent lighter after calving. The Lewisburg 2-year-olds lost only 40 pounds from the before-calving weight to the after-calving weight—equivalent to 5.5 per cent of the latter. The Beltsville Jersey shrinkage was 8 per cent. Only 12 of the 20 reached their before-calving weights in the tenth month of lactation.

In the twelfth month their average weight was 836 pounds and the average gain over the after-calving weight was 118.7 pounds or 16 per cent.

They lost very slightly in the first, second, fourth and sixth months—the total average loss was only 21 pounds. Actually they just about held their weight during the first 6 months, the total average gains of 20 pounds balancing the total average loss of 21 pounds, but starting with the seventh month they gained consistently during the remainder of the lactation period. This in contrast with the Beltsville 2-year-old Jerseys that gained from the first month to the twelfth.

The Lewisburg heifers were experiencing breeding trouble. Only half of them conceived during the lactation period, but this does not appear to have materially affected the results so far as gains are concerned. The 10 heifers that did not conceive made an average gain from after calving to the twelfth month of 118 pounds, while the 10 heifers that carried calves an average of 176 days during the lactation period made average gains of 122 pounds. The average butterfat yield was not materially affected by pregnancy; the 10 that carried calves an average of 176 days had an average yield of butterfat of 393.6 pounds and those that did not conceive had an average yield of 385.2 pounds.

The weights of the mature Beltsville Jerseys behaved in a somewhat different manner than did those of the 2-year-olds. The mature cows had an average before-calving weight of 1,201 pounds, 79 pounds or approximately 7 per cent greater than their after-calving weight. This is only 1 per cent less than the shrinkage of the 2-year-olds. Only four cows attained their precalving weights in the tenth month. In the twelfth month all had passed their after-calving weights, but their gain was only 6.6 per cent of the after-calving weight, approximately one third the percentage gain made by the Beltsville 2-year-olds. Their losses in weight during the lactation period were more pronounced than those of the 2-year-olds. All but two had lower weights than the after-calving weight during the lactation period. The low points were very scattered, two of them occurring as late as the eighth and the ninth months of the lactation period. After the first month the Beltsville 2-year-olds showed an average gain each month, while the mature Jerseys, with the exception of the second month, when they showed a loss, just about held the average after-calving weight through the

seventh month of lactation. Starting with the eighth month they showed a material gain each month.

THE HOLSTEINS

The average before-calving weight of the Beltsville 2-year-old Holsteins, 1,238 pounds, was almost 12 per cent greater than their after-calving average. Like the 2-year-old Jerseys they made a gain in weight each month, excepting the first, which was the same as the after-calving weight. Only three of them dropped below their after-calving weight at any point in the lactation period. Three of them failed to attain their before-calving weights in the tenth month, and all of them passed their after-calving weights by substantial margins by the twelfth month of lactation. These gains ranged from 95 to 367 pounds, and the average gain of 234 pounds was 21 per cent of the average after-calving weight. This is a larger percentage gain than that made by the Beltsville 2-year-old Jerseys.

Twenty 2-year-old Holstein heifers at the Huntley, Mont., Station had an average before-calving weight of 1,279 pounds, 13.7 per cent greater than their after-calving average. Both the before-calving and the after-calving average weights were somewhat higher than those of the Beltsville 2-year-olds. However, the system of feeding at Huntley did not enable them to gain as consistently during the lactation period as did the 2-year-olds at Beltsville under the system of feeding prevailing at that station. Only 5 of the 12 heifers attained their before-calving weights in the tenth month of lactation, and 17 of the 20 fell below their after-calving weights during the lactation period. On the average the Huntley heifers were below their after-calving weight for the first three months of the lactation. The low weight during the lactation for the 17 heifers showing a loss ranged from 15 to 220 pounds. However, by the twelfth month they had all passed their after-calving weights, the average gain at that time being 180.5 pounds or 16 per cent of the after-calving average. This is less than the percentage gain of the Beltsville 2-year-olds by some 5 per cent. Actually the Huntley heifers made an average total gain during the lactation period of 215 pounds, as compared to an average gain of 234 pounds by the Beltsville 2-year-olds, but the Huntley heifers had an average loss of 30 pounds in the first month to overcome, while the Beltsville heifers had no loss. The Huntley heifers were heavier than the Beltsville heifers at the start of the lactation period by an average of 19 pounds, but they were lighter in the twelfth month of lactation by an average of 28 pounds.

The mature Holstein cows at Beltsville had an average weight before-calving of 1,719 pounds, 8.4 per cent more than their after-calving weight. This is a smaller shrinkage due to calving than was experienced by the Holstein 2-year-olds at Beltsville and Huntley.

On the basis of averages these 9 mature cows remained remarkably close

to their after-calving weight during the first, third, and fourth months of lactation. In the second month they dropped 15 pounds below their after-calving weight. From the fifth to the eighth months of lactation their average weights only ranged from 1,607 to 1,611 pounds, but during the remaining 4 months they made consistent gains each month. In the tenth month, only one cow had regained her before-calving weight. In the twelfth month all had gained over their after-calving weights, the average gain of 111 pounds being an increase of 7 per cent. Thus the per cent gain over the after-calving weight in the mature Holsteins was only about one third as great as for the 2-year-old Beltsville heifers. This is almost the same relationship as was shown by the relative gains of the mature Jersey cows to those of the 2-year-olds.

Seven of the 9 Holstein cows had low points in their weights that ranged from 2 to 103 pounds below their after-calving weights. Four of the 7 cows reached the lowest level of weight in the second month, one in the third month, one in the fourth and one in the seventh month of lactation.

CONCLUSIONS

1. The shrinkage from the before-calving to the after-calving weights, on the basis of the per cent of the after-calving weight, was greater in the Holsteins than in the Jerseys and greater in the 2-year-olds than in the mature cows. The greatest shrinkage was in the Huntley Holstein 2-year-olds—13.7 per cent—and the least in the Lewisburg Jersey 2-year-olds—5.5 per cent. The smaller Jersey calf weight accounts for a part of the difference between the Holstein and Jersey breeds.

2. In total average gains from the after-calving weight to the twelfth month of lactation, expressed as per cent of the after-calving weight, the Beltsville 2-year-old Jerseys and 2-year-old Holsteins gained 18.4 and 21 per cent, respectively, and the Beltsville mature Jersey cows and the Beltsville mature Holstein cows gained 6.6 and 7 per cent, respectively. These four groups were under the same system of feeding—requirements plus 10 per cent. It appears that the 2-year-olds—Jersey and Holsteins—made from 2.5 to 3 times the gains of the mature cows. The Lewisburg 2-year-old Jerseys and the Huntley 2-year-old Holsteins had the same average percentage gain—16 per cent. The system of feeding these two groups, while the usual full grain feeding system, is somewhat less liberal than the requirements plus 10 per cent.

3. Under the system of feeding followed at the Beltsville Station the average weights of both the 2-year-old Jerseys and the 2-year-old Holsteins were approximately the same in the first month of lactation as their after-calving weights, but thereafter they made gains each month. The gains were not as rapid in the early months as in the last months of the lactation period but on the average there were no losses. The mature Jerseys and the mature

Holsteins under the same system of feeding showed a different pattern of gains from those of the 2-year-olds but were remarkably similar to each other. The Jersey cows had an average loss of 78 pounds in the second month but gained it back in the third.   In the first 7 months of lactation they lost 82 pounds and gained 90 pounds on the average, so that they were just about holding even.   In the last 5 months they had an average gain of 68 pounds. The Holsteins had an average loss of 20 pounds in the second month.   In the first 7 months they lost an average of 22 pounds and gained an average of 45, so they were a little more than holding even.   In the last 5 months their average gain was 89 pounds.   The Lewisburg 2-year-old Jerseys held an even weight the first 6 months and made consistent gains the last 6 months. The Huntley 2-year-old Holsteins had an average loss of 30 pounds the first month, and gained each month thereafter.

4. It appears that, with the requirements-plus-10-per-cent system of feeding, 2-year-olds will gain from the first month of lactation, and with mature cows the average gains will somewhat more than balance the losses in the first 7 months and rapid gains will occur during the balance of the lactation. Under the usual full-grain system of feeding, that is 1 pound of grain to each 3 pounds of milk produced, the average losses and gains balanced each other in the first 6 months and consistent gains occurred during the balance of the lactation with 2-year-old Jerseys, and with 2-year-old Holsteins after an average loss the first month, there were gains on the average each month during the balance of the lactation period.

FROZEN HOMOGENIZED MILK. II. EFFECT OF FREEZING AND STORAGE TEMPERATURES ON THE CHEMICAL AND BACTERIOLOGICAL PROPERTIES OF HOMOGENIZED MILK

LIEUTENANT COLONEL C. J. BABCOCK, Sanitary Corps, AUS, MAJOR RICHARD
N. ROERIG, Sanitary Corps, AUS, CAPTAIN JOSEPH N. STABILE,
Sanitary Corps, AUS, CAPTAIN WILLIAM A. DUNLAP,
Sanitary Corps, AUS, AND COLONEL RAYMOND
RANDALL, Veterinary Corps, USA.

*Army Veterinary School, Medical Department Professional Service Schools,
Army Medical Center, Washington, D. C.*

In part I, "Effect of Freezing and Storage Temperature on the Physical Characteristics of Homogenized Milk" (1), it was shown that freezing and storage temperatures affect the physical character of homogenized milk. To determine if these conditions cause chemical changes which may contribute to the physical changes, a study was made of the shift of the chemical constituents in samples of homogenized milk frozen and stored under the same conditions as the samples used to study the physical changes. Bacterial counts were also made on a number of the samples to determine whether biological changes occur in frozen homogenized milk.

In a study on frozen homogenized milk, Cvitl (2) analyzed the outer layer, the part which froze first, as well as the top, middle and bottom portions of the remainder of the sample and found that the central portion was richer in fat, casein, albumin, globulin, sugar, and chloride ion than the upper or lower portions and that the outer layer was the poorest in these constituents. Baldwin and Doan (3) reported that when milk, whose creaming ability was destroyed by heat or homogenization, was frozen, the fat concentration of the unfrozen portion increased progressively with the extent of freezing, while that of the frozen portion decreased at first, but finally approached the fat percentage of the original milk as the extent of freezing approached 100 per cent. Trout (4) reported that when homogenized milk was frozen and then thawed a marked settling of the fat and solids-not-fat was noted. The lower 15 per cent of creaming cylinders of slowly thawed frozen homogenized milk contained as high as 7.7 per cent fat and 24.60 per cent total solids in contrast with 2.0 per cent and 5.50 per cent, respectively, in the upper 15 per cent layer. Fabian and Trout (5) found that from a bacteriological standpoint there is no reason why clean, wholesome, fresh cream cannot be pasteurized and stored for a period of 1 year in glass, paper, or tin containers at temperatures ranging from $-5°$ to $-10°$ F.

Sections of the homogenized milk were obtained for analysis by dividing the quart samples, while frozen, either into thirds, or into halves.

The following methods of analysis were used:

a. Protein—Kjeldahl-Gunning-Arnold Method using the factor of 6.38.
b. Fat—Mojonnier Method.
c. Total solids—A.O.A.C. Method.
d. Lactose—Munson-Walker Method.
e. Chloride ion—Volhard Method using the milliequivalent value of 0.00355.
f. Ash—Heated in a muffle furnace at a temperature not higher than 525° C.
g. Bacteria—The bacteria content of the milk was determined by the standard plate count in accordance with the method as outlined in Standard Methods for the Examination of Dairy Products, APHA, 8th edition, 1941.   The plates were incubated at 37° C.

EXPERIMENTAL RESULTS

Chemical

The results of the chemical analyses of aliquots from different sections of quart samples of homogenized milk, frozen in paper containers of one quart capacity and stored under various conditions, are shown in table 1. The analyses of two samples of the homogenized milk prior to freezing gave the following average values: protein, 3.24 per cent; fat, 3.72 per cent; total solids, 12.26 per cent; lactose, 4.77 per cent; and chloride, 0.095 per cent.

Table 1 shows that when homogenized milk was frozen and stored in the frozen state the solid constituents were more concentrated in the bottom section.   When the quart samples were divided into two equal parts the lower halves contained a larger proportion of the milk solids.   Analyses of the frozen samples after divisions into three equal parts disclosed the fact that the highest concentration of milk solids was in the bottom third, the next highest concentration was in the middle third, and the lowest was in the top third.   It may also be seen from table 1 that the analyses of the middle third were in good agreement with those of unfrozen milk, as well as samples of milk which had been frozen and stored and then thawed.   Those slight discrepancies which occurred may be attributed to experimental error, especially in samples showing separation.

The data in table 1, A, were obtained by the analysis of milk samples frozen and held at −10° C. (14° F.) for 21 to 100 days, at −32.8° C. (−27° F.) for 30 to 109 days, and at −40° C. (−40° F.) for 51 to 82 days.   The analyses of the individual samples indicated that the freezing and storage temperatures as well as the storage time may influence the distribution of the milk solids between the various sections of the frozen milk.

TABLE 1

Chemical analyses of different sections of homogenized milk frozen in quart paper containers and stored under various conditions

No. samples	Section analyzed	% Protein	% Fat	% Total solids	% Lactose	% Chlorides	% Ash
A. Frozen and stored at constant temperature (−10° C., −32.8° C., −40° C.)							
8	Top third	2.55	2.77	9.85	3.85	0.077	0.58
	Middle third	3.17	3.48	12.27	4.81	0.095	0.74
	Bottom third	3.66	4.23	13.94	5.45	0.104	0.82
6	Top half	3.09	3.16	10.88	4.11	0.093	....
	Bottom half	3.88	3.91	13.65	5.13	0.116	....
11	Whole quart	3.35	3.64	12.66	4.79	0.096	0.73
B. Frozen and held at −32.8° C., or −10° C., followed by storage at a lower temperature (−40° C. or −32.8° C.)							
6	Top half	3.03	3.03	10.71	3.99	0.093	....
	Bottom half	4.00	3.92	13.94	5.20	0.117	....
C. Frozen and held at −40° C., −32.8° C., or −10° C., followed by storage at a higher temperature (−32.8° C., −10° C., or −3° C.)							
10	Top third	2.25	2.56	8.77	3.33	0.067	0.51
	Middle third	3.14	3.30	12.15	4.67	0.093	0.73
	Bottom third	3.98	4.04	15.17	6.07	0.115	0.90
6	Top half	3.16	3.33	11.21	4.14	0.093	....
	Bottom half	3.80	3.93	13.51	4.99	0.117	....
D. Frozen and held at −32.8° C. or −10° C., exposed to room temperature (23° C.) for ½ hour, then stored at −10° C.							
24	Top third	2.37	2.39	9.44	3.49	0.070	0.53
	Middle third	3.15	3.14	12.06	4.61	0.094	0.69
	Bottom third	3.75	3.74	14.24	5.56	0.107	0.81
2	Top half	2.95	2.99	10.46	4.00	0.091	....
	Bottom half	3.97	3.94	13.91	5.35	0.117	....
E. Frozen and held at −32.8° C. or −10° C., exposed to room temperature (23° C.) for 1 hour, then stored at −10° C.							
24	Top third	2.53	2.52	9.97	3.84	0.076	0.61
	Middle third	3.08	2.71	12.33	4.83	0.095	0.73
	Bottom third	3.36	3.42	13.58	5.15	0.099	0.78
2	Top half	3.04	3.13	10.98	4.20	0.095	....
	Bottom half	3.84	3.80	13.38	5.01	0.112	....
F. Frozen and held at −32.8° C. or −10° C., exposed to room temperature (23° C.) for 2 hours, then stored at −10° C.							
24	Top third	2.51	2.61	9.82	3.64	0.070	0.54
	Middle third	2.83	2.87	12.04	4.62	0.093	0.67
	Bottom third	3.66	3.40	14.12	5.47	0.106	0.82
2	Top half	2.87	2.90	10.23	3.85	0.088	....
	Bottom half	3.97	3.87	13.96	5.29	0.120	....
G. Frozen and held at −32.8° C. or −10° C., exposed to room temperature (23° C.) for 4 hours, then stored at −10° C.							
24	Top third	1.83	2.09	7.58	2.72	0.054	0.50
	Middle third	2.81	2.75	11.42	4.33	0.084	0.70
	Bottom third	4.47	3.92	17.69	7.01	0.136	0.99
2	Top half	1.83	1.88	6.56	2.40	0.056	....
	Bottom half	4.93	4.58	17.32	6.57	0.145	....

Table 1, B, presents the data obtained when two samples of homogenized milk were frozen at − 32.8° C. (− 27° F.) then moved to − 40° C. (− 40° F.); two frozen at − 10° C. (14° F.) then moved to − 32.8° C.; and two frozen at − 10° C. then moved to − 40° C. The milk was held at the initial freezing temperature for 9 days and at the lower temperature for 54 to 80 days prior to analysis. The results given in table 1, A and B, show that lowering the storage temperature of frozen homogenized milk did not significantly affect the distribution of milk solids.

As indicated in table 1, C, some of the samples were frozen at − 40° C. (− 40° F.) and moved to − 32.8° C. (− 27° F.) or to − 10° C. (14° F.); some were frozen at − 32.8° C. then moved to − 10° C. or to − 3° C. (26.6° F.); and others were frozen at − 10° C. and moved to − 3° C. The samples were held for an average of 3 days at the lower temperature and for an average of 70 days at the higher temperature prior to analysis. A comparison of the results in C with those in A and B (table 1) indicates that moving frozen milk to a higher storage temperature does not materially affect the results of the chemical analyses of the different sections of the sample.

The data presented in table 1, E, F, G, and H, were obtained by the analysis of samples which were exposed to room temperature for various

TABLE 2

Effect of freezing and storage temperatures on the standard plate count of homogenized milk

No. samples	Average storage time (days)	Average standard plate count per ml.
A. Frozen and held at constant temperature (− 40° C., − 33° C., or − 10° C.)		
3	56	11,000
3	88	12,000
B. Frozen and stored at a constant temperature (− 10° C.) then moved to a lower temperature (− 33° C.)		
1	59	15,000
1	92	20,000
C. Frozen and stored at a constant temperature (− 33° C. or − 40° C.) and then moved to a higher temperature (− 10° C. or − 33° C.)		
3	53	2,000
3	85	3,000
D. Frozen and stored at a constant temperature (− 33° C. or − 10° C.) exposed to room temperature, then stored at − 10° C.		
2 (exposed ½ hr.)	59	4,000
2 (exposed ½ hr.)	92	7,000
2 (exposed 1 hr.)	59	3,000
2 (exposed 1 hr.)	92	4,000
2 (exposed 2 hrs.)	59	3,000
2 (exposed 2 hrs.)	92	6,000
2 (exposed 4 hrs.)	59	3,000
2 (exposed 4 hrs.)	92	6,000

lengths of time between storage at two different freezing temperatures. These conditions were intended to simulate those which occur when frozen milk is moved from storage to a freezer on a ship. A comparison of the results shown in E, F, G, and H with each other and with those in C demonstrates that such conditions did not affect the distribution of milk solids unless the frozen milk was exposed to room temperature for four hours. After four hours there was a considerable increase in the concentration of milk solids in the lower section and a corresponding decrease in the upper section.

Bacteriological

Standard plate counts were performed on samples of milk prior to freezing and again after freezing and storage under different conditions in order to determine whether these environments permit multiplication of bacteria. The average plate count of the milk prior to freezing was 28,000 per ml. Table 2 shows the average plate count after the milk had been frozen and stored.

The data in table 2 indicate that freezing milk and storing it in the frozen state had a tendency to lower the number of bacteria per milliliter as determined by the standard plate count and further that the bacterial content was not materially affected by changes in storage time and temperature, or by exposure to room temperature for four hours.

Results obtained on individual samples indicated that the count was not influenced by the freezing or storage temperatures.

SUMMARY

It has been found that when homogenized milk is frozen the solid components tend to concentrate in the lower portions of the sample. This distribution was not materially affected by changes in freezing and storage temperature. Exposure of the frozen milk to room temperature (23° C.) less than four hours did not alter the distribution of the milk solids, but at that time there was a significant shift of the solid components toward the lower portion.

Changes in the temperature at which frozen homogenized milk was stored did not materially affect the chemical composition of the different sections of the quart samples.

The chemical analyses of the various sections of frozen homogenized milk were not affected by exposure to room temperature, 23° C. (73.8° F.), unless the milk was exposed for four hours. When the milk was exposed for four hours there was a considerable increase in the percentage of milk solids in the bottom third and a considerable decrease in the percentage of the constituents in the top third of quart samples.

Homogenized milk that was frozen and subsequently thawed at room temperature (23° C.) changed in physical character. The degree of change

was dependent upon the freezing and storage temperature and the length of storage. However, these conditions did not cause any significant change in the chemical character of the milk, indicating that the chemical character of frozen homogenized milk does not contribute to the physical changes.

Freezing and storing homogenized milk in the frozen state had a tendency to lower the number of bacteria per milliliter as determined by the standard plate count. This decrease was not materially affected by freezing and storage temperatures, by changes in the storage temperature, or by exposure to room temperature for four hours.

REFERENCES

(1) BABCOCK, C. J., *et al*. Effect of Freezing and Storage Temperature on the Physical Characteristics of Homogenized Milk. JOUR. DAIRY SCI., 29: 699–706. 1946.
(2) CVITL, JOSEFA. Einfluss des Gefrierens auf einige Bestandteile und Eigenschaften der Milch. Milchw. Forsch, 12: 409–432. 1931.
(3) BALDWIN, F. BRUCE, JR., AND DOAN, F. J. Observations on the Freezing of Milk and Cream. JOUR. DAIRY SCI. 18: 629–637. 1935.
(4) TROUT, G. M. The Freezing and Thawing of Milk Homogenized at Various Pressures. JOUR. DAIRY SCI. 24: 277–287. 1941.
(5) FABIAN, F. W., AND TROUT, G. M. Influence of Various Treatments on the Bacterial Content of Frozen Cream. JOUR. DAIRY SCI. 26: 959–965. 1943.

TOXICITY OF PHENOTHIAZINE DERIVATIVES EXCRETED IN THE MILK OF DAIRY COWS TREATED WITH MASSIVE DOSES OF THE DRUG*

GEORGE H. WISE,[1] C. A. JAMES, III,[2] AND G. W. ANDERSON[3]

*Dairy Department, South Carolina Agricultural Experiment Station,
Clemson College, Clemson, S. C.*

The effectiveness of phenothiazine as an anthelmintic has resulted in its widespread and, in some cases, indiscriminate administration to animals. In recent years the recognition of the toxic reactions of this drug has led to increased caution in its dosage. Stewart (8), in a review of the subject, indicated that man and the bovine are the most susceptible species to phenothiazine poisoning, and that the young are less resistant than the adults. Hence, it would appear that the infant might be highly sensitive to this drug.

Portions of phenothiazine derivatives are excreted in the milk of lactating ewes (9) and goats (6) following medication. Though milk contaminated with these derivatives usually is diverted from food channels, occasionally it is offered inadvertently for human consumption (1), thus constituting a potential health hazard.

Adult cattle ordinarily are not heavily infested with internal parasites, but the unthrifty condition of a cow in areas where parasitic infestation is common may lead to phenothiazine therapy. The possibility that milk from a cow treated with this drug might be fed to infants presented a problem warranting investigation. In this study single massive doses of phenothiazine were given to individual lactating cows for the purpose of ascertaining: the clinical effects on the cows, the period of elimination of the phenothiazine derivatives in the milk, and the toxic effects of the contaminated milk on young rats.

EXPERIMENTAL

Effects of massive doses of phenothiazine on lactating dairy cows. A representative of each of three breeds, Guernsey, Holstein, and Jersey, were used in this investigation. Pertinent data on the experimental subjects are presented in table 1.

The cows were in an excellent state of health. They were subjected to standard managerial and feeding practices; the rations, consisting of a

TABLE 1

Experimental cows that received massive doses of phenothiazine

Breed	Age	Body weight	Stage of gestation	Stage of lactation
	years	lbs.	days	days
Guernsey	7	1055	78	210
Holstein	5	1065	154	182
Jersey	8	990	51	174

concentrate mixture, beet pulp and either hay or silage, were fed twice daily, at the milking periods.

Each cow was dosed with 125 grams of commercial phenothiazine, which amount was approximately twice the maximum recommended for adult cattle. Observations during the four days following medication revealed no changes except a precipitous drop in the milk production of two of the cows and a slight decrease in that of the third. The milk yields persisted at the lowered level for two to three days but subsequently returned to the pre-treatment level for the Holstein and the Jersey cows, the higher producers, but remained low for the Guernsey.

Abnormal red coloration was observed on the udders of the cows the day following the administration of the drug. This, presumably, was the result of oxidation of the phenothiazine conjugates (5, 9) in the urine that had come in contact with the surface of the udder.

Period of excretion of phenothiazine derivatives in the milk. In accordance with common practices the cows were milked at 12-hour intervals. Samples of milk from the individual animals were collected at each milking for a period of 72 hours following administration of the drug.

Determinations of the presence of phenothiazine derivatives were made by qualitative methods described by Collier (5). Acidification of the milk samples with strong hydrochloric acid, producing a mauve color, was the most satisfactory test used. Exposure of the samples to air and light for several hours resulted in the development of a pink color in the serum phase of the milk. This color was difficult to detect in samples having a high carotenoid content; the yellow seemed to mask the pink. In addition to the procedures indicated by Collier (5), it was found that mixing "Aerosol",

TABLE 2

Period of elimination of phenothiazine derivatives in milk following medication

Breed	Hours after dosing					
	12	24	36	48	60	72
Guernsey	+++	+++	++	+	+	−
Holstein	+++	++	+	−	−	−
Jersey	+++	+++	++	+	+	−

a surface-tension-reducing reagent, with milk containing the conjugates of phenothiazine produced a transitory pink color, which was adequate for detection but unsatisfactory for estimating the degree of concentration.

As shown in table 2, the phenothiazine products were in the milk for periods of 36 to 60 hours after dosing. The highest concentrations apparently were in the first milking following administration.

Toxicity of phenothiazine derivatives excreted in the milk of the cows. Samples of milk collected from the individual cows at the 12-hour and the 24-hour periods were fed to young rats to ascertain whether any toxic reactions would be evinced. Immediately after the collections, half of each sample was stored in the raw state at 35° F., but the other half was boiled for three to five minutes (recommended treatment of milk for infant feeding) before storing.

Thirteen month-old rats, grouped as indicated in table 3, were restricted to diets of the various milk samples for 72 hours. During a preliminary

TABLE 3

Rates at which young rats consumed various samples of milk containing phenothiazine derivatives

Samples of milk fed		No. of rats	Av. wt. of rats	Daily consumption per 100 grams of body wt.	
Period of collection	Treatment			Average	Range
			grams	*cc.*	*cc.*
12-hour	None	3	55	70	63–78
	Boiled	3	55	74	72–79
24-hour	None	3	52	74	71–80
	Boiled	3	55	71	63–83
Daily (herd)	Pasteurized	1	57	77	....

adjustment period of 60 hours, the individual rats were fed pasteurized whole milk *ad lib.* The pasteurized milk diet was replaced by the experimental samples, a fresh supply being provided twice daily.

The rate of consumption of the milk samples, as shown in table 3, reveals a marked individual variation but no significant group differences. Evidently the presence of the phenothiazine conjugates in the milk did not change its palatability. Clinical examinations of the rats during and after the milk feeding period revealed no discernible toxic effects.

DISCUSSION

In accord with the report of Britton (4), the phenothiazine derivatives eliminated in the milk seemed to have a preservative value. The milk samples containing the conjugates were in excellent condition after storage for 31 days at 35° F. Furthermore, as noted by Swales and Collier (9), samples exposed to light and air in a warm room for several days showed no

evidence of decomposition. These properties, either bacteriostatic or bactericidal, have been ascribed to the oxidation product, thionol (4). The microbiological phase of the subject merits further investigation.

Boiling the milk for several minutes apparently modified neither its physiological effects on rats nor its qualitative reactions to various agents. The effects of extensive boiling, producing a concentration of the products, were not investigated. This type of treatment, however, is beyond the limits of practical measures used in the home preparation of milk for direct consumption.

Although phenothiazine derivatives are eliminated in the milk of cows treated with the drug, the concentration of these conjugates from recommended therapeutic doses probably is too small to cause serious toxic reactions in the consumer. No ill effects have been demonstrated in pigs (2), kids (6) and lambs (9) that have consumed milk from their respective dams treated with the commonly prescribed doses of phenothiazine. In recovery trials with sheep Swales and Collier (9) observed that between 80 and 85 per cent of a therapeutic dose of phenothiazine was eliminated in the feces and urine; thus only a small percentage could be excreted in the milk.

Though it is hazardous to attempt to translate experimental findings, particularly in toxicity studies, from young rats to infants, the results of this investigation suggest that milk from cows given a therapeutic dose of phenothiazine is not likely to be toxic to the normal human subject. The cows in this experiment were given twice the recommended amount, which should have produced a concentration of derivatives in the milk at least as great as the maximum from the standard doses given to any other adult cattle. Furthermore, the rats, though more resistant than the human being (8), consumed quantities of milk per unit of weight from five to six times greater than normally would be fed to babies (7).

The element of human variation in susceptibility to phenothiazine, as indicated in results reported by Bercovitz et al. (3), warrants adherence to the general recommendation that milk from cows treated with the drug either be discarded or be used for purposes other than human consumption during the period that the derivatives are excreted. This introduces the problem of determining whether or not the conjugates are in the milk. Since the pink color that develops upon exposure to air frequently is difficult to detect in milk having highly pigmented fats, this procedure, though practical, cannot be regarded as an infallible indicator of the absence of the derivatives. Therefore, as a precautionary measure, it probably is well to divert the milk from food channels for a period of two to three days following medication.

SUMMARY

1. Oral administration of 125 grams of commercial phenothiazine, over twice the maximum recommended therapeutic dose, to healthy adult lactat-

ing cows produced no detectable deleterious effects other than a temporary repression of milk production.

2. Derivatives of phenothiazine were detected in the milk for periods of 36 to 60 hours following administration of the drug.

3. Young rats restricted to diets of the milk collected at the 12-hour and the 24-hour periods after dosage manifested no discernible symptoms of toxicity.

REFERENCES

(1) ANONYMOUS. Colored Milk. Dairy Goat Jour., 24(1): 16–17. 1946.

(2) ANDREWS, J. S., AND CONNELLY, J. W. The Value of Phenothiazine for the Removal of Nodular Worms from Pregnant and Nursing Sows. Helminthol. Soc. Wash., Proc., 11(1): 13–15. 1944.

(3) BERCOVITZ, Z., PAGE, R. C., AND DE BEER, E. J. Phenothiazine: Experimental and Clinical Study of Toxicity and Anthelmintic Value. Amer. Med. Assoc. Jour., 122(15): 1006–1007. 1943.

(4) BRITTON, J. W. The Materia Medica of Phenothiazine. Cornell Vet., 31(1): 1–12. 1941.

(5) COLLIER, H. B. The Fate of Phenothiazine in the Sheep. Canad. Jour. Res., Sect. D, Zool. Sci., 18(7): 272–278. 1940.

(6) HABERMANN, R. T. The Effect of Phenothiazine on Pregnant Goats and their Offspring. Vet. Med., 38(3): 96–99. 1943.

(7) LENROOT, K. F. Infant Care. Children's Bureau, U. S. Dept. Labor, Pub. 8, p. 81. 1943.

(8) STEWART, M. A. Phenothiazine in Veterinary Practice. Jour. Amer. Vet. Med. Assoc., 106(817): 217–222. 1945.

(9) SWALES, W. E., AND COLLIER, H. B. Studies on Effects and Excretion of Phenothiazine when Used as an Anthelmintic for Sheep. Canad. Jour. Res., Sect. D, Zool. Sci., 18(7): 279–287. 1940.

A DEVICE TO AID IN DETERMINING THE EFFECTIVENESS OF DAIRY DETERGENTS

E. L. FOUTS AND T. R. FREEMAN

Florida Agricultural Experiment Station, Gainesville, Florida

A wide variety of water types is found in Florida. Because of the widespread limestone deposits, waters of widely varying degrees of hardness are encountered. Also, because of the proximity of the Atlantic Ocean and the Gulf of Mexico considerable concentrations of salt are found in the water in certain areas. A project is in progress to determine the most suitable washing powders to use with the various types of water. It is not the purpose of this paper to report the results of the experiments on washing
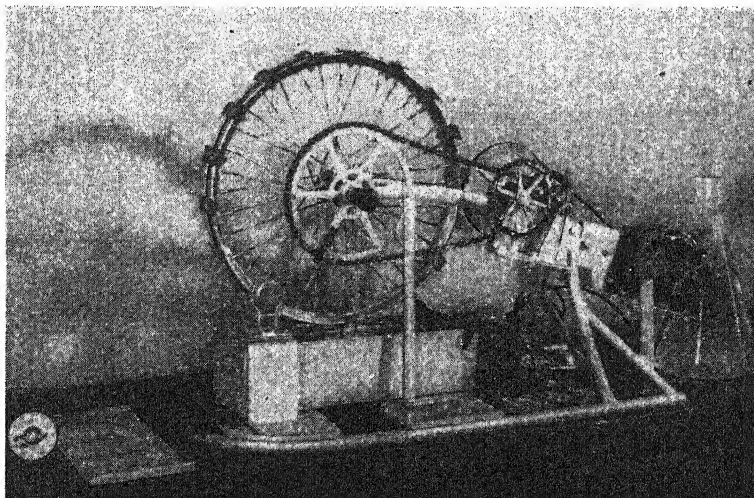
FIG. 1. Deterg-O-Meter—An apparatus designed to test the efficiency of dairy cleaners. Glass slides with a baked-on milk film are fastened onto the rim of the wheel by means of metal clips. The wheel is then lowered into water at 50° C. containing the washing powder to be tested. The wheel turns slowly, dipping the slides into the water and then rubbing them against the sponge rubber brush. The average number of revolutions required to clean the slides gives an index of the efficiency of the cleaner.

powders but to describe the apparatus being used to aid in determining the effectiveness of various washing powders in cleaning dairy equipment.

The wide scope of this problem suggested the need for a mechanical device to aid in making washing trials. Since no such device was available, one was built. It has been named the Deterg-O-Meter. It consists of a 16-inch metal bicycle wheel mounted in a pivoted frame which permits vertical movement, powered by an electric motor and geared to turn at 7 r.p.m. Evenly

TABLE 1

Method of determining the "soil removal index"

Washing powder No. 1

Revolutions	Trial 1 — Slide number		Trial 2 — Slide number		Trial 3 — Slide number	
40	1, 13	80	14	40	5, 6	80
50	2, 3, 4, 6, 8, 9, 10, 12, 16	450	1, 8, 9, 12, 17, 2, 10, 11	400	2, 4, 8, 9, 10, 11, 13	350
60	5, 11, 14, 17, 18	300	5, 6, 7, 13, 16, 18	360	1, 3, 12, 14, 16, 17, 18	420
70	7	70	3, 4	140	7	70
		900		940		920

$$\frac{900}{17} = 52.9 \qquad \frac{940}{17} = 55.3 \qquad \frac{920}{17} = 54.1$$

$$\frac{52.9 + 55.3 + 54.1}{3} = 54.1 \text{ soil removal index}$$

Washing powder No. 2

Revolutions	Trial 1 — Slide number		Trial 2 — Slide number		Trial 3 — Slide number	
20	18	20	1, 2, 7, 12, 13, 16	120	11, 12	40
30	1, 2, 5, 6, 7, 11, 12, 13, 14, 15, 16	330	8, 10, 11, 14, 17, 18	180	1, 3, 4, 6, 9, 13, 14, 15	240
40	3, 4, 9	120	4, 5, 6, 9	160	2, 5, 7, 10, 16, 17	240
50	8, 10	100	3, 15	100	18	50
60					8	60
		570		560		630

$$\frac{570}{17} = 33.5 \qquad \frac{560}{18} = 31.1 \qquad \frac{630}{18} = 35.0$$

$$\frac{33.5 + 31.1 + 35.0}{3} = 33.2 \text{ soil removal index}$$

Washing powder No. 3

Revolutions	Trial 1 — Slide number		Trial 2 — Slide number		Trial 3 — Slide number	
30	4	30	7	30		
40					12	40
50					13	50
60	9, 11	120	1, 2, 6, 12	240	2, 9, 11	180
70	5, 8, 10, 12, 18	350	9, 11, 13, 15	280	1, 3, 6, 10, 14, 17	420
80	2, 3, 7, 14, 17	400	5, 8, 10, 14, 17	400	15, 18	160
90	6, 13, 16	270	16, 18	180	4, 7	180
100	1	100			8	100
110			4	110		
120	15	120			5	120
130						
140			5	140		
		1390		1380		1250

$$\frac{1390}{18} = 77.2 \qquad \frac{1380}{18} = 76.6 \qquad \frac{1250}{17} = 73.5$$

$$\frac{77.2 + 76.6 + 73.5}{3} = 75.7 \text{ soil removal index}$$

spaced around the rim of the wheel are 18 numbered metal clips, which hold securely 18, 3 by 1 inch glass microscope slides.

Milk films are prepared on one side of the clean slides by spreading 0.4 ml. of whole milk evenly and uniformly over a rectangular area $\frac{3}{4}$ by $1\frac{1}{8}$ inches. The slides then are heated in an oven for 3 hours at 120° C. which produces a tough hard film which is very resistant to cleaning.

Four liters of the water to be tested containing a weighed amount of the washing powder to be tested are warmed to 50° C. and poured into the pan. The slides are slipped under the numbered clips and the wheel is lowered so that when it revolves it dips into the water. The temperature of the water is maintained at 50° C. by means of a hot plate under the water bath. Immediately after dipping into the water, the slides rub against the sponge rubber brush. As the slides become clean they are removed from the wheel and a record is made of the number of revolutions required to remove the film. The average number of revolutions required to clean the 18 slides is determined.

Second and third runs are made and if these 3 runs check within 5 revolutions, the average of the 3 runs (54 slides) is accepted as being accurate. If the first 3 trials fail to check within 5 revolutions, additional runs are made until satisfactory checks are obtained. The brush is made of sponge rubber refrigerator door gasket material and a new brush is installed after each 20 runs. The data shown in table 1 illustrate the procedure used to calculate the "soil removal index." Other tests also are being made to aid in determining which powders are most suitable for washing dairy equipment in the various types of Florida water.

ASSOCIATION ANNOUNCEMENT

NOMINATING COMMITTEE

The nominating committee consists of the following members:

Chairman: A. C. RAGSDALE, University of Missouri, Columbia, Missouri

J. A. NELSON, Montana State College, Bozeman, Montana

I. W. RUPEL, Texas A. & M. College, College Station, Texas

FLOYD JOHNSTON, Iowa State College, Ames, Iowa

H. W. NORTON, Holstein-Friesian Ass'n of America, Brattleboro, Vermont

The Constitution and By-laws of the American Dairy Science Association provides for the publication of the names of the nominating committee in the January issue of the JOURNAL OF DAIRY SCIENCE. It is the duty of this Committee, of which **A. C. Ragsdale** is the present chairman, to send its report to the secretary not later than April 1. The secretary will then send out the ballots to secure a final vote before the annual meeting. The results of the election will be announced at the annual meeting.

As a mmber of the Association, it is your obligation to express your wishes for officers and directors to any member of this Committee. Two candidates for vice-president and four for directors will be nominated. The present vice-president automatically becomes president.

The Nominating Committee will give consideration to the wishes of the members as expressed by correspondence and also will consider additional candidates. They have been instructed to study the list of past officers and directors (see pages 803 and 804 of the August, 1943, Journal) to plan to secure good geographic distribution and to recognize the desirability of representation from all lines of activity of our members.

This change in the Constitution was made so that our Association could elect its officers in a democratic way. This can only be done, however, if our members are prompt in giving their opinions freely to the Nominating Committee.

OFFICERS AND COMMITTEES OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

ASSOCIATION OFFICERS

FORDYCE ELY, Kentucky, *President*

P. H. TRACY, Illinois, *Vice President*

R. B. STOLTZ, Ohio, *Secretary-Treasurer*

ASSOCIATION COMMITTEES
BORDEN AWARDS COMMITTEES

Manufacturing

K. G. WECKEL, Wisconsin, *Chairman*

B. E. HORRAL, Indiana

BEN GOODALE, South Carolina

Production

J. B. FITCH, Minnesota, *Chairman*

C. W. TURNER, Missouri

G. M. CAIRNS, Maryland

RESOLUTIONS COMMITTEE

W. E. KRAUSS, Ohio, *Chairman*

K. L. TURK, New York

J. H. HILTON, North Carolina

G. H. WILSTER, Oregon

GLENN VERGERONT, Wisconsin

NOMINATING COMMITTEE

A. C. RAGSDALE, Missouri, *Chairman*

J. A. NELSON, Montana

I. W. RUPEL, Texas

FLOYD JOHNSTON, Iowa

H. W. NORTON, Vermont

PROGRAM COMMITTEE

J. A. NELSON, Montana, *Chairman*

C. L. HANKINSON, Minnesota

W. T. CRANDALL, New York

D. M. SEATH, Louisiana

Ontario Agricultural College Representative

JOURNAL MANAGEMENT COMMITTEE

GEORGE E. HOLM, Washington, D. C., *Chairman*

W. E. PETERSEN, Minnesota

F. E. NELSON, Iowa, Ex-officio

T. S. SUTTON, Ohio

R. B. STOLTZ, Ohio, Ex-officio

HONORS COMMITTEE

A. C. DAHLBERG, New York, *Chairman*

R. B. BECKER, Florida

H. S. WILLARD, Wyoming

AUDITING COMMITTEE

O. F. GARRETT, Ohio, *Chairman*

RICHARD A. LARSON, Ohio   W. E. KRAUSS, Ohio

NECROLOGY COMMITTEE

E. S. GUTHRIE, New York, *Chairman*

R. B. BECKER, Florida   H. S. WILLARD, Wyoming

OFFICERS AND COMMITTEES OF THE MANUFACTURING SECTION
SECTION OFFICERS

C. L. HANKINSON, Minnesota, *Chairman*

PAUL R. ELLIKER, Indiana, *Vice-Chairman*   J. M. TRIMBLE, Indiana, *Secretary*

MANUFACTURING SECTION COMMITTEES
Standing Committee on Milk

E. O. HERREID, Illinois, *Chairman*

HENRY TRANMAL, Illinois   D. V. JOSEPHSON, Ohio

Standing Committee on By-Products

H. C. JACKSON, Wisconsin, *Chairman*

J. B. STINE, Illinois   A. O. CALL, Illinois

Standing Committee on Dried Milk

G. C. SUPPLEE, New York, *Chairman*

ARNOLD H. JOHNSON, Maryland   A. D. HOLMES, Massachusetts

Standing Committee on Cheese

P. R. ELLIKER, Indiana, *Chairman*

C. A. PHILLIPS, California   A. B. ERICKSON, Wisconsin

Standing Committee on Evaporated Milk

B. H. WEBB, Washington, D. C., *Chairman*

D. V. JOSEPHSON, Ohio   E. B. OBERG, Wisconsin

Standing Committee on Butter

G. H. WILSTER, Oregon, *Chairman*

D. K. HOWE, Nebraska   C. JENSEN, North Dakota

Standing Committee on Ice Cream

H. H. SOMMER, Wisconsin, *Chairman*

C. A. IVERSON, Iowa   W. C. COLE, California

Standing Committee on Products Judging

G. M. Trout, Michigan, *Chairman*

P. A. Downs, Nebraska   E. L. Fouts, Florida

C. J. Babcock, Washington, D. C.   E. O. Anderson, Connecticut

Standing Committee on Program

B. E. Horrall, Indiana, *Chairman*

F. J. Babel, Iowa   W. C. Loy, Illinois

Temporary Committee on Reorganization and Records

S. T. Coulter, Minnesota, *Chairman*

D. V. Josephson, Ohio   R. Whitaker, Maryland

OFFICERS AND COMMITTEES OF THE PRODUCTION SECTION

SECTION OFFICERS

D. M. Seath, Louisiana, *Chairman*

G. H. Wise, Kansas, *Vice-Chairman*   L. A. Moore, Maryland, *Secretary*

PRODUCTION SECTION COMMITTEES

Program Committee

D. M. Seath, Louisiana, *Chairman*

G. H. Wise, Kansas   G. W. Salisbury, New York

Breeds Relations Committee (Joint with Extension Section)

Production Section

H. A. Herman, Missouri, *Chairman*

K. L. Turk, New York   W. W. Yapp, Illinois

Extension Section

Lynn Copeland, Tennessee   E. C. Scheidenhelm, New Jersey

Floyd Johnston, Iowa

Dairy Cattle Breeding Committee (Joint with Extension Section)

Production Section

E. E. Heiser, Wisconsin   L. O. Gilmore, Minnesota

J. L. Lush, Iowa

Extension Section

E. J. Perry, New Jersey, *Chairman*

C. L. Blackman, Ohio   S. J. Brownell, New York

Dairy Cattle Health Committee (Joint with Extension Section)

Production Section

D. M. Seath, Louisiana   L. A. Moore, Maryland

W. E. Peterson, Minnesota

Extension Section

C. G. BRADT, New York, *Chairman*

J. W. LINN, Kansas
A. I. MANN, Connecticut

OFFICERS AND COMMITTEES OF THE EXTENSION SECTION

With the exception of the Program Committee, all committee members are appointed for a period of three years.

The Program Committee is automatically appointed by the election of the officers of the Extension Section: It consists of the Chairman, Vice-Chairman, Secretary, and an Extension Representative from the host state.

Committees made up of five members retire one member every third year, and at the two intervening years two men each are retired. Vacancies caused by the expiration of a term are filled by three-year appointments. All appointments expire immediately following the annual meeting in the year designated.

In addition to five committees made up of members entirely in the Extension Section there are three joint committees with the Production Section. These committees have three men represented from each section and each year one man retires from each sectional group. The chairman of these committees is selected jointly by the two section chairmen.

To be a member of a committee an extension dairyman must be a member of the American Dairy Science Association.

PROGRAM COMMITTEE

Chairman—W. T. CRANDALL, Cornell University, Ithaca, New York

E. H. LOVELAND, University of Vermont, Burlington, Vermont

A. R. PORTER, Iowa State College, Ames, Iowa

Appointment from host state to be announced later

EXHIBIT COMMITTEE

	Term Expires
Chairman—To be appointed from host state	1949
L. A. JOHNSON, Michigan State College, East Lansing, Michigan	1947
J. A. AREY, University of North Carolina, Raleigh, North Carolina	1947
D. L. MURRAY, Michigan State College, East Lansing, Michigan	1948
I. E. PARKIN, Pennsylvania State College, State College, Pennsylvania	1948

BREED RELATIONS COMMITTEE

(Members appointed by Production Section)

Chairman—H. A. HERMAN, University of Missouri, Columbia, Missouri 1949

K. L. TURK, Cornell University, Ithaca New York 1947

W. W. YAPP, University of Illinois, Urbana, Ill. 1948

(Members appointed by Extension Section)

LYNN COPELAND, University of Tennessee, Knoxville, Tennessee 1947

E. C. SCHEIDENHELM, Rutgers University, New Brunswick, New Jersey 1948

FLOYD JOHNSTON, Iowa State College, Ames, Iowa 1949

DAIRY CATTLE BREEDING COMMITTEE

(Members appointed by Extension Section)

Chairman—E. J. PERRY, Rutgers University, New Brunswick, N. J.   1949

C. L. BLACKMAN, Ohio State University, Columbus, Ohio   1947

S. J. BROWNELL, Cornell University, Ithaca, N. Y. 1948

(Members appointed by Production Section)

E. E. HEISER, University of Wisconsin, Madison, Wisconsin 1947

L. O. GILMORE, University of Minnesota, St. Paul, Minnesota 1948

J. L. LUSH, Iowa State College, Ames, Iowa 1949

DAIRY CATTLE HEALTH COMMITTEE

(Members appointed by Extension Section)

Chairman—C. G. BRADT, Cornell University, Ithaca, N. Y. 1947

J. W. LINN, Kansas State College, Manhattan, Kansas 1948

A. I. MANN, University of Connecticut, Storrs, Connecticut 1949

(Members appointed by Production Section)

D. M. SEATH, Louisiana State University, Baton Rouge, Louisiana 1947

L. A. MOORE, Bureau of Dairy Industry, Washington, D. C. 1948

W. E. PETERSEN, University of Minnesota, St. Paul, Minnesota 1949

DAIRY RECORDS COMMITTEE

Chairman—J. F. KENDRICK, Bureau of Dairy Industry, Washington,
    D. C. 1947
    W. T. CRANDALL, Cornell University, Ithaca, N. Y. 1947
    C. R. GEARHART, Pennsylvania State College, State Col-
    lege, Pennsylvania 1948
    A. R. PORTER, Iowa State College, Ames, Iowa 1948
    E. J. STALLARD, University of Wisconsin, Madison, Wis-
    consin 1949

4-H CLUB COMMITTEE

Chairman—G. W. VERGERONT, University of Wisconsin, Madison,
    Wisconsin 1947
    IVAN McKELLIP, Ohio State University, Columbus, Ohio 1947
    J. C. NAGEOTTE, Pennsylvania State College, State Col-
    lege, Pennsylvania 1948
    GEORGE McINTYRE, U. P. Experiment Station, Chatham,
    Michigan 1948
    H. A. WILLMAN, Cornell University, Ithaca, N. Y. 1949

OFFICERS OF THE EXTENSION SECTION

Chairman—W. T. CRANDALL, Cornell University, Ithaca, N. Y.
Vice-Chairman—E. H. LOVELAND, University of Vermont, Burlington, Ver-
    mont
Secretary—A. R. PORTER, Iowa State College, Ames, Iowa
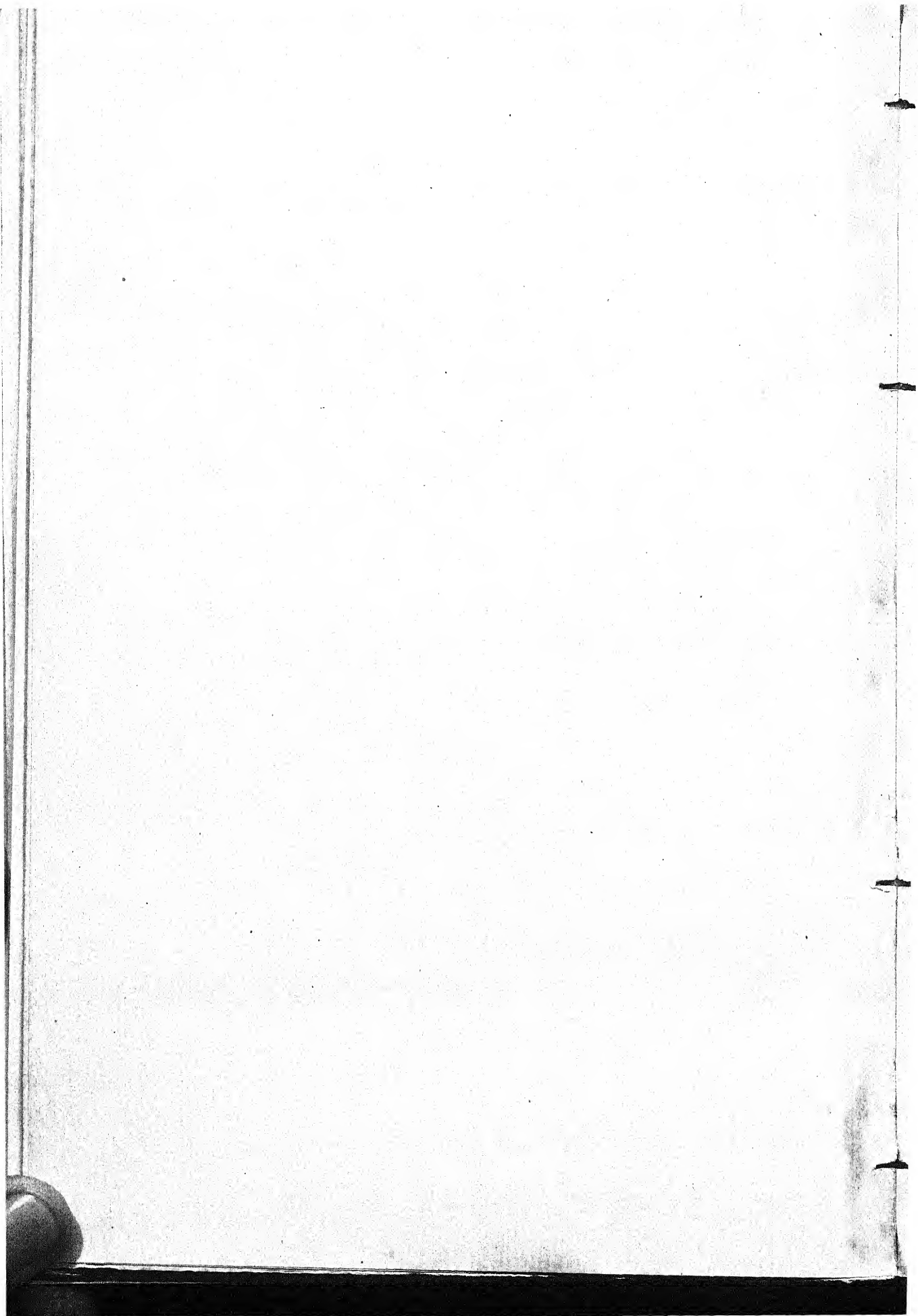
THE THIAMINE, RIBOFLAVIN, NICOTINIC ACID AND PANTO-THENIC ACID CONTENTS OF MARE'S COLOSTRUM AND MILK AND ASCORBIC ACID CONTENT OF THE MILK

P. B. PEARSON

Nutrition Laboratory, Agricultural Experiment Station and School of Agriculture, Agricultural and Mechanical College of Texas, College Station, Texas

The importance of colostrum to the new-born animal has been emphasized by various investigators, and it is generally believed that new-born animals having access to colostrum do better than those that are fed only milk. The work of Lundquist and Phillips (8) suggests that some of the B vitamins play an important role in the prevention of certain diseases of the new-born calf. The work of Gamble, Earle, and Howe (4) emphasizes the importance of the proteins of colostrum for foals. While there is extensive literature on the vitamin content of milk of various species, information on the various B vitamins in colostrum has become available but recently and then only for colostrum of the human (2, 14, 15) and the cow and ewe (10). These studies show that colostrum differs from milk in respect to various members of the B vitamin group.

The present study was undertaken to provide information on the thiamine, riboflavin, nicotinic acid, and pantothenic acid contents of mare's colostrum as compared with mare's milk. Information on the B vitamins of various species is of interest for comparative purposes and possibly in the preparation of substitutes for feeding orphaned foals.

Information on the ascorbic acid content of mare's milk is very limited, and the two values found in the literature differ by about ten-fold. Holmes and associates (6) recently reported ascorbic acid values for mare's milk ranging from 0.6 to 2.3 mg. per 100 ml., while the ascorbic acid values reported by Cimmino (1) ranged from 8.7 to 19.7 mg. per 100 ml. of milk. The magnitude of the differences in the reported ascorbic acid content of mare's milk is too great to be accounted for on the basis of diet or breed differences. Therefore, it seemed desirable to obtain additional information on the ascorbic acid content of mare's milk.

EXPERIMENTAL

The mares used for this study represented various breeds of light horses. No attempt was made to control the dietary regimen of the mares. All were

fed essentially the same except for the amount of green feed, which depended on season and availability of pasturage. Since riboflavin (12) and possibly other (13) of the B vitamins are known dietary essentials, diet may be a factor in the concentration in the colostrum and milk of some of the vitamins studied.

The colostrum was collected within 12 hours after foaling. All the milk samples were from mares that had been nursing foals for 30 days or longer. The samples were collected into brown bottles, and precautions were taken against photochemical destruction of any of the vitamins.

Thiamine was determined by the thiochrome method of Hennessy (5). Riboflavin was determined by the microbiological method (16) on the autoclaved sample following filtration at a pH of approximately 4.6 to remove the proteins and foreign growth stimulants. Nicotinic acid was determined by the microbiological method of Krehl, Strong, and Elvehjem (7), with the slight modification devised by Pearson and Leucke (11) which was found to be satisfactory for colostrum and milk. Pantothenic acid was determined by the method of Neal and Strong (9), following the enzymatic liberation of the vitamin by takadiastase and papain. The Evelyn photoelectric method (3) described for urine was adapted to the determination of the reduced ascorbic acid in milk by precipitating the proteins with an equal volume of 10 per cent trichloroacetic acid.

RESULTS AND DISCUSSION

Samples of colostrum were collected from eight mares and samples of milk from fifteen mares. Milk samples were collected from all of the animals from which colostrum was obtained except for mare number 1. The values for thiamine, riboflavin, nicotinic acid, and pantothenic acid expressed in micrograms per 100 ml. of colostrum are shown in table 1, and the values for milk in table 2. The values for reduced ascorbic acid per 100 ml. of milk are shown in table 3. Some of the milk samples for the ascorbic acid studies were obtained from animals not appearing in tables 1 or 2. The mares used for the ascorbic acid studies have, therefore, been designated by letter in order to avoid confusion with animals in the first two tables.

Thiamine. The average thiamine content of mare's colostrum was 38 μg. per 100 ml. as compared with 16 μg. per 100 ml. of milk. The fact that the thiamine content of the milk is lower than the value for colostrum is in accord with observations for the cow and ewe (10). The average thiamine content of cow's colostrum is 62 μg. per 100 ml., and of the ewe 108 μg. per ml. as compared with 38 μg. per 100 ml. for mare's colostrum. The corresponding average values for milk of the respective species are 38, 60, and 16 μg. per 100 ml. The average thiamine value for mare's milk reported in this paper is somewhat lower than the values reported for four animals by Holmes *et al* (6).

TABLE 1

Thiamine, riboflavin, nicotinic acid, and pantothenic acid content of mare's colostrum
(Values in µg. per 100 ml.)

Mare number	Thiamine	Riboflavin	Nicotinic acid	Pantothenic acid
1	65	162	155	700
2	40	135	167	750
3	37	112	114	800
4	21	125	150	900
5	34	125	185	425
6	34	120	178	400
7	38	165	200	950
8	40	160	134	1050
Average ...	38	138	160	747

Riboflavin. The average riboflavin content of mare's colostrum was 138 µg. per 100 ml. as compared with 40 µg. per 100 ml. for the milk. The fact that mare's colostrum is much richer than the milk in riboflavin is in accord with the observations on the cow and ewe (10), as cow's colostrum contains more than three times as much riboflavin as does the milk, while in the case of the ewe the difference is still greater. It is also of interest that the riboflavin contents of the colostrum and milk of the mare are much lower than the values for the cow and ewe. Mare's colostrum contains an average of 138 µg. per 100 ml. as compared with 610 µg. per 100 ml. of cow's colostrum and 2008 µg. per 100 ml. of ewe's colostrum; the corresponding values for the milk are 40, 177, and 436 µg. per 100 ml.

Nicotinic acid. The average nicotinic acid content of mare's colostrum was 160 µg. per 100 ml. as compared with 58 µg. per 100 ml. of milk. The nicotinic acid values reported here for mare's milk are slightly lower than values reported previously for four mares (6). The nicotinic acid value for mare's colostrum is higher than the value that has been reported (10) for cow's colostrum, but not as high as the value for ewe's colostrum.

TABLE 2

Thiamine, riboflavin, nicotinic acid, and pantothenic acid content of mare's milk
(Values in µg. per 100 ml.)

Mare number	Thiamine	Riboflavin	Nicotinic acid	Pantothenic acid
2	22	37	50	400
3	12	36	40	250
4	22	45	55	300
7	26	36	68	330
8	10	42	60	350
9	10	38	70	350
10	12	47	60	510
11	10	30	50	390
12	12	30	50	230
13	18	70	60	225
14	20	32	70	315
15	15	40	70	320
Average	16	40	58	331

Pantothenic acid. The pantothenic acid value of mare's colostrum is much higher than has been reported for the colostrum of other species. The average pantothenic acid content of mare's colostrum was 747 µg. per 100 ml. as compared with values of 224 and 262 µg. per 100 ml. for the colostrum of the cow and ewe, respectively. The average value of 331 µg. of pantothenic acid per 100 ml. of mare's milk is of the same order as values previously reported (6) for three animals. At present there is no physiological explanation for the high pantothenic acid level of mare's colostrum or for the fact that in this species the colostrum contains significantly more pantothenic acid than the milk, whereas cow's or ewe's milk contains more pantothenic acid than does the colostrum.

Ascorbic acid. The reduced ascorbic acid was determined on samples of milk collected from eight mares. These mares had been in milk for 2 or more months. From table 3 it will be seen that the values for reduced ascorbic acid ranged from 9.26 to 14.46 mg. per 100 ml. of milk, with an average of 11.83 mg. This figure is approximately ten times the value reported

TABLE 3

The reduced ascorbic acid content of mare's milk

Mare number	mg./100 ml.
A	13.62
B	9.26
C	11.95
D	14.46
E	12.45
F	12.95
G	9.96
H	9.96
Average	11.83

by Holmes and associates (6), but it agrees reasonably well with the values ranging from 8.7 to 19.7 mg. per 100 ml. of milk which were reported by Cimmino (1).

As a check on our analytical procedure we determined the reduced ascorbic acid in samples of milk from eight cows. The values for cow's milk ranged from 1.3 to 2.2 mg. per 100 ml. These figures agree well with numerous values reported for cow's milk. Thus mare's milk contains about five times as much ascorbic acid as cow's milk.

SUMMARY

Studies were made of the thiamine, riboflavin, nicotinic acid, and pantothenic acid contents of mare's colostrum and milk and of the reduced ascorbic acid content of mare's milk.

Mare's colostrum was found to contain an average of 38 µg. of thiamine, 138 µg. of riboflavin, 160 µg. of nicotinic acid and 747 µg. of pantothenic acid per 100 ml. The corresponding values for mare's milk are 16 µg. of thiamine, 40 µg. of riboflavin, 50 µg. of nicotinic acid, and 331 µg. of panto-

thenic acid per 100 ml. Mare's colostrum contains significantly less thiamine and riboflavin and more pantothenic acid than cow's colostrum. Cow's milk is richer in each of the four vitamins than mare's milk.

The average value for reduced ascorbic acid for mare's milk was 11.8 mg. per 100 ml. This is approximately five times greater than the ascorbic acid values found in this laboratory and reported in the literature for cow's milk.

REFERENCES

(1) CIMMINO, AIDO. Vitamin C in Horse Milk. Annali d'igiene, 50: 471–472. 1940. (Chem. Abs., 38: 1780. 1944.)

(2) CORYELL, M. N., HARRIS, M. E., MILLER, S., WILLIAMS, H. H., AND MACY, I. G. Nicotinic Acid, Pantothenic Acid and Biotin Contents of Colostrum and Mature Human Milk. Amer. Jour. Dis. Children, 70: 150–161. 1945.

(3) EVELYN, K. A., MALLOY, H. T., AND ROSEN, C. The Determination of Ascorbic Acid in Urine with the Photoelectric Colorimeter. Jour. Biol. Chem., 126: 645–654. 1938.

(4) GAMBLE, J. A., EARLE, I. P., AND HOWE, P. E. Rearing Foals and Lambs by the Use of Colostrum, Blood Serum, and Substitute Milks. U. S. Dept. Agr. Tech. Bul. 661. 1938.

(5) HENNESSY, D. J. A Standard Thiochrome Assay for the Determination of Thiamine in Cereal Products. Cereal Chem. Bul. 2, No. 2. 1942.

(6) HOLMES, A. D., MCKEY, B. V., WERTZ, A. W., LINDQUIST, H. G., AND PARKINSON, L. R. The Vitamin Content of Mare's Milk. Jour. Dairy Sci., 29: 163–171. 1946.

(7) KREHL, W. A., STRONG, F. M., AND ELVEHJEM, C. A. Determination of Nicotinic Acid. Modifications in the Microbiological Method. Indus. and Engin. Chem., Analyt. Ed., 15: 471–475. 1943.

(8) LUNDQUIST, N. S., AND PHILLIPS, P. H. Certain Dietary Factors Essential for the Growing Calf. Jour. Dairy Sci., 26: 1023–1030. 1943.

(9) NEAL, A. L., AND STRONG, F. M. Microbiological Determination of Pantothenic Acid. Indus. and Engin. Chem., Analyt. Ed., 15: 654–657. 1943.

(10) PEARSON, P. B., AND DARNELL, A. L. The Thiamine, Riboflavin, Nicotinic Acid and Pantothenic Acid Content of Colostrum and Milk of the Cow and Ewe. Jour. Nutr., 31: 51–57. 1946.

(11) PEARSON, P. B., AND LUECKE, R. W. The B Vitamin Content of Raw and Cooked Sweet Potatoes. Food Res., 10: 325–329. 1945.

(12) PEARSON, P. B., SHEYBANI, M. K., AND SCHMIDT, H. Riboflavin in the Nutrition of the Horse. Arch. Biochem., 3: 467–474. 1944.

(13) PEARSON, P. B., SHEYBANI, M. K., AND SCHMIDT, H. The B Vitamin Requirements of the Horse. Jour. Anim. Sci., 3: 166–174. 1944.

(14) RODERUCK, C. E., CORYELL, M. N., WILLIAMS, H. H., AND MACY, I. G. Free and Total Riboflavin Contents of Colostrum and Mature Human Milk. Amer. Jour. Dis. Children, 70: 171–175. 1945.

(15) RODERUCK, C. E., WILLIAMS, H. H., AND MACY, I. G. Free and Total Thiamine Contents of Colostrum and Mature Human Milk. Amer. Jour. Dis. Children, 70: 162–170. 1945.

(16) SNELL, E. E., AND STRONG, F. M. A Microbiological Assay for Riboflavin. Indus. and Engin. Chem., Analyt. Ed., 11: 346–350. 1939.

A STUDY OF HEAT TOLERANCE IN JERSEY COWS

R. F. GAALAAS[1]

*Bureau of Dairy Industry, Agricultural Research Administration,
U. S. Department of Agriculture*

In a previous article, the author (1) reported that there was a strong correlation between air temperature and body temperature of Jersey cows when the air temperatures were above 75° F., and that there was a wide variaton in the body reaction of different cows under the same conditions. Rhoad (4, 5) has found this to be true also for beef cattle and has proposed using the body temperature (converted to a heat-tolerance coefficient for convenience) under standardized conditions as one of the criteria for judging the suitability of cattle for areas having considerable periods of high air temperatures (2, 3). Seath and Miller (6) analyzed data on Jersey and Holstein cows in Louisiana and found that relative humidity plays a minor rôle in comparison to air temperature as a factor influencing body temperature, respiration rate, and pulse rate.

The author has not found, in the published data on the characteristics of the heat tolerance of individual animals, answers to the following questions: (1) Is the body temperature of the individual sufficiently stable from year to year to be a sound measure of the heat tolerance of the animal, or, in other words, is the heat-tolerance coefficient a fixed individual characteristic, such as fat percentage? (2) Do the age of the animal, the stage of lactation, and the stage of gestation affect the body reaction? (3) Are there real differences in reaction between individuals and groups of cows? These questions are of considerable importance to the livestock industry in the southern United States and in tropical countries, where the production of both beef and dairy products is low. This study was made in an attempt to answer them.

EXPERIMENTAL METHODS

The methods of gathering the data have been described in a previous report (1). For this study only the body temperatures in the afternoons for the 4-month period of June through September of each year were used. The report covers the five summer periods of 1941 through 1945. All body temperatures used in this report were obtained in the barn, and the cows were grazed on pastures in which shade was available during the test periods of each year.

The heat-tolerance coefficient for each cow was determined by first calculating the body temperature at 90° F. air temperature from linear regres-

sion equations and then converting this body temperature to a heat-tolerance coefficient by use of the equation $HT = 100 - [14(BT - 101)]$. BT represents the calculated body temperature and HT the heat-tolerance coefficient. This equation is a modification of the one devised by Rhoad (2, 3) and is based on the assumption that a body temperature of $108°+$ at $90°$ F. air temperature and under otherwise normal conditions would indicate complete loss of control in the regulation of body temperature, or zero per cent efficiency in eliminating surplus body heat. Conversely, a body temperature of $101°$ F. is considered as normal, and a cow that could maintain that body temperature at $90°$ F. air temperature would be considered 100 per cent efficient in eliminating surplus body heat. The heat-tolerance coefficient is used rather than the body temperature as it is a simpler figure to work with and gives a better expression of the degree of heat tolerance than does the body temperature.

An air temperature of $90°$ F. is used as a base for determining the body reaction as the maximum air temperature is $90°$ F. or higher on about 67 per cent of the 122 days in the June-through-September period at this station. Cattle, therefore, must be able to withstand $90°$ F. temperatures continuously if they are to be considered as well adapted to this area.

Since there is a known regression of body temperature on air temperature, it was considered that calculating the body temperature at $90°$ F. from regression formulae would be a more accurate method of determining the average body temperature for each animal than using the average body temperature within a specified air temperature range. This method also permits the use of all readings made during the test period and tends to eliminate differences of air temperature from year to year. The number of readings used in calculating the regression coefficient for each cow each year ranged from 10 to 18, with an average of 16.8 readings being used for determining each heat-tolerance coefficient.

One hundred and thirty-seven heat-tolerance coefficients were determined for 74 different cows during the 5-year period. Due to unavoidable circumstances, there was a rapid turnover in the herd during the period under study, and heat-tolerance coefficients were obtained on many of the cows for one year only and on only a few cows for more than 2 years.

EXPERIMENTAL RESULTS

Table 1 lists the number of cows included each year, the average heat-tolerance coefficient for the herd, the highest and lowest heat-tolerance coefficients for an individual in each year, and the range of air temperature encountered during the summer periods. An analysis of variance showed the differences in the yearly means of the heat tolerance to be not significant statistically. (The analyses of variance in this report were made by methods described by Snedecor (7). The term "significant" or "*"

TABLE 1

Heat-tolerance data of all cows, by years

Year	Number of cows	Heat-tolerance coefficients (%)*			Air temperatures (°F.)†		
		Average	Maxi-mum	Mini-mum	Average	Maxi-mum	Mini-mum
1941	21	77.8	90	62	86.6	94	78
1942	34	76.1	92	61	86.1	95	77
1943	20	81.2	92	65	88.4	96	80
1944	29	79.2	90	68	90.1	98	79
1945	33	77.6	89	64	85.9	93	77
Total or average	137	78.1	92	61	87.4	98	77

* The maximum is the highest for any individual cow; the minimum, the lowest for any individual cow; the average is that for all the cows.

† The figures given are the average for the four-summer-months periods of June through September and the highest and lowest temperatures encountered while taking the readings in the barn.

means that "F" values were between the 5 and 1 per cent points; and the term "highly significant" or "**" means that the "F" values were larger than those required at the 1 per cent level.)

Since there seemed to be little difference in the average heat tolerance of the herd from year to year, the data were grouped by age of the cow at the time of determining the coefficient, with the results shown in table 2.

There would seem to be a real difference between the average heat tolerance of the different age groups, since the "F" value is greater than that required for significance at the 1 per cent level. Apparently, there is a definite increase in heat tolerance as the cows increase in age from 2 to 3 years and probably some decrease again at ages beyond 8 years. An analysis of variance of the yearly means for each group was made, and the differences were found to be not significant from year to year except for the 3-year-old group. There was a wide variation from year to year in this 3-year-old group, and the "F" value for the between-year variance was greater than required for significance at the 1 per cent level. A possible explanation for this difference will be referred to under sire group analyses.

TABLE 2

Heat-tolerance data of all cows, by age groups

Age group*	Number of cows	Heat-tolerance coefficients		
		Average	Maximum	Minimum
6 years and over	27	75.8	92	61
5 years to 5 years 11 months	17	79.0	89	64
4 years to 4 years 11 months	28	79.5	92	65
3 years to 3 years 11 months	38	80.8	92	69
2 years to 2 years 11 months	27	74.7	90	61
Total or average	137	78.1	92	61

* Age of each cow was figured on August 1 of each year (center of test period).

TABLE 3

Correlation of heat-tolerance coefficients

Simple correlation (r) between heat-tolerance coefficients of cows determined at:	r	Number of cows
2 years of age and same cows at 3 years of age	+ 0.24	19
3 years of age and same cows at 4 years of age	− 0.21	21
2 years of age and same cows at 4 years of age	+ 0.47	10
4 years of age and same cows at 5 years of age	+ 0.57*	11
5 years of age and same cows at 6 years of age	+ 0.96**	7
6 years of age or over and next consecutive year	+ 0.84*	5
4 years of age or over and next consecutive year	+ 0.66**	23

*. Near to significance as determined from table 7.2 of Snedecor (7).
** Highly significant as determined from table 7.2 of Snedecor (7).

To measure the stability of the heat-tolerance coefficient for the individual cow from year to year, correlations were calculated, with the results shown in table 3.

It is obvious from these results that the heat-tolerance coefficient determined at 2 or 3 years of age would have little value in predicting the heat-tolerance coefficient of an animal at 4 years or more of age. Thus, while there is a definite increase in average heat tolerance from 2 to 3 years of age, as noted above, the increase is not at all uniform among the individuals studied. The heat-tolerance characteristic does appear to be more stable in the older cows. The coefficients of correlation between the heat-tolerance determinations made at 4 and 5 years of age, and between consecutive years at ages above 6 years, approach the 5 per cent level of significance, and the heat-tolerance determinations made at 5 and 6 years of age are almost perfectly correlated. The last group of table 3 is composed of all cows having heat-tolerance coefficients for two consecutive years, made at ages of 4 years or more. The correlation coefficient is higher than that required for statistical significance at the 1 per cent level, indicating that among the older cows there is a reasonable degree of stability in the heat-tolerance characteristic of the individual from one year to the next.

A considerable difference in heat tolerance of individual cows under similar conditions has been regularly noted in the studies here, and an analysis of variance was made to determine if these differences are great enough to be significant. Since the heat tolerance is unstable at the younger

TABLE 4

Analysis of variance of data on body temperatures of individual cows 4 years old and over

Source of variance	df	Sum of squares	Mean square
Total	1208	808.49	
Between-individual-cow means	71	242.01	3.409**
Within individual cow	1137	566.48	0.498

** Highly significant.

ages, only those cows were used which had determinations made at 4 years of age or older.

Analyses first were made for each of the five test periods, and the mean square of the between-cow variation was found to be highly significant in every year, the "F" values ranging from one and one-half to four times that required for significance at the 1 per cent level. The analysis of variance of the data for 5 years is given in table 4. There would seem to be no question but that there is a real difference in the body response of the individual cows to the climatic factors at this station.

To determine the effect of lactation and gestation on the heat-tolerance coefficient, the data from three groups of cows were studied. Group I cows were milking approximately one-half of a summer period and dry the balance of the same period and were pregnant 180 days or more at the start of the dry period. Group II cows were milking approximately one-half of

TABLE 5

Comparison of heat tolerance of cows when milking and dry

Group number*	Number of cows	Average heat tolerance		Average days pregnant—start of period	
		Milking	Dry	Milking	Dry
I	18	75.5	77.6	71.8	211.0
II	5	85.8	87.4	61.8	107.2
III	6	76.5	78.8	13.8	100.5

* Group I were in milk approximately half of a summer period and dry the balance of the period and were pregnant 180 days or more at start of the dry period.

Group II were in milk approximately half of a summer period and dry the balance of the period and were pregnant less than 180 days at the start of the dry period. Heat tolerance averages for the milking and dry periods for Groups I and II are, therefore, for the same cows in the same year.

Group III were milking all of one summer period and dry all of the previous or subsequent summer period. Heat tolerance averages for the milking and dry periods are, therefore, for the same cows in different years. Cows that were 4 years old or over in the first year included are the only ones included in this group.

a summer period and dry the balance of the same period but were pregnant less than 180 days at the start of the dry period. Group III cows were milking all of one summer period and dry all the previous or subsequent summer period. The averages are shown in table 5.

The differences between the average heat tolerance of the cows when milking and when dry are small in all three groups and cannot be considered as significant, since the mean square for within-group variance is larger than that for between milking and dry in each case. Apparently, there is little difference in body reaction regardless of the stage of lactation or gestation.

Since there was a definite difference in the reaction of individual cows, it would be desirable to know if this difference is inherited. A comparison of sire groups is given in tables 6 and 7. Sufficient data are not yet available to attempt to determine the influence of the dam.

R. F. GAALAAS

TABLE 6

Comparison of heat-tolerance coefficients of groups of daughters of different sires

| | Heat-tolerance coefficients of daughters determined at: | | | | | |
| Sire* | 2 years of age | | 3 years of age | | 4 years or more of age | |
	No. of daus.	Average *HT*	No. of daus.	Average *HT*	No. of daus.	Average *HT*
VGM	......	....	......	....	4	81.8
571	......	....	......	....	8	79.0
137	......	....	5	86.4	4	80.8
715	6	70.2	7	75.3	11	80.0
82	8	78.9	12	81.8	7	78.7
140	6	70.2	10	81.6	5	78.0
60	5	78.4	......	....	......	....

* Only those sires having four or more daughters with *HT* determinations are included.

The averages and the analyses of the sire groups are made on heat-tolerance coefficients determined at 2, 3, and 4 years of age and over, separately, since it was shown above that the heat-tolerance coefficients are not stable in the younger cows.

The difference between the mean heat tolerances of the sire groups appears to be fairly definite for the determinations made at 2 and 3 years of age, since the "*F*" values for these two mean squares fall between the 5 and 1 per cent points, but there is apparently no appreciable difference between the sire-group means where the determinations were made at 4 years or more of age. The influence of the sire on the heat tolerance of his daughters would, therefore, appear to be negligible, except at the younger ages.

In the analysis of the yearly means for various age groups (see table 2) it was noted that the means of the 3-year-old group varied significantly from year to year. This is believed due to the fact that in 1941 this group was made up entirely of daughters of 137, with a high average heat tolerance; and in 1942 of daughters of 715, with a low average heat tolerance (see

TABLE 7

Analysis of variance of data on heat-tolerance of sire groups

Group	Source of variance	*df*	Sum of squares	Mean square
I *HT* determined at 2 years of age	Total Between sire groups Within sire groups	24 3 21	1,406 454 952	.... 151.33* 45.33
II *HT* determined at 3 years of age	Total Between sire groups Within sire groups	33 3 30	1,299 387 912	.... 129.00* 30.40
III *HT* determined at 4 years or more of age	Total Between sire groups Within sire groups	38 5 33	1,586 47 1,539	.... 9.40 46.64

* Significant at 5% level.

table 6). In the other 3 years, the 3-year-old groups were made up of daughters of two or more sires, and the average heat tolerances were not much different from the 5-year average. This is believed to be the only place in this study where the sire influence would bias the results, as all of the other groups (except in tables 6 and 7) are composed of daughters of several different sires.

CONCLUSIONS

The following conclusions would appear to be warranted for Jersey cows under conditions prevailing at this station:

1. Not much change in the average heat tolerance of the herd occurs from year to year.

2. A definite difference in heat tolerance in the different age groups exists with the 2-year-olds showing the lowest average and the 3-year-olds the highest average.

3. The heat-tolerance coefficient is a reasonably stable individual characteristic at ages of 4 years and above, but not at 2 or 3 years of age.

4. There is a real difference in physiological response of different cows to the same environmental conditions, as measured by the body temperature.

5. The stage of lactation and gestation has little, if any, effect on the heat-tolerance coefficient.

6. There is some difference in the response of groups of daughters of various sires when measured at 2 and 3 years of age but little, if any, when measured at 4 years of age or more.

ACKNOWLEDGMENT

REFERENCES

(1) GAALAAS, R. E. Effect of Atmospheric Temperature on Body Temperature and Respiration Rate of Jersey Cattle. Jour. Dairy Sci., 28(7): 555–563. 1945.

(2) RHOAD, A. O. A Scale of Heat Tolerance. Jour. Anim. Sci., 1(2): 85. 1942.

(3) RHOAD, A. O. The Iberia Heat Tolerance Test for Cattle. Trop. Agr., 21(9): 162–164. 1944.

(4) RHOAD, A. O. Some Observations on the Response of Purebred Bos taurus and Bos indicus Cattle and Their Crossbred Types to Certain Conditions of the Environment. Amer. Soc. Anim. Prod. Proc., 31: 284–295. 1938.

(5) RHOAD, A. O. A Method of Assaying Genetic Differences in the Adaptability of Cattle to Tropical and Subtropical Climates. Empire Jour. Expt. Agr., 8(31): 190–198. 1940.

(6) SEATH, D. M., AND MILLER, G. D. The Relative Importance of High Temperature and High Humidity as Factors Influencing Respiration Rate, Body Temperature, and Pulse Rate of Dairy Cows. Jour. Dairy Sci., 29(7): 465–472. 1946.

(7) SNEDECOR, GEO. W. Statistical Methods. Collegiate Press, Ames, Ia. 1938.

SYNTHETIC RATIONS FOR THE DAIRY CALF[1]

A. C. WIESE,[2] B. CONNOR JOHNSON, H. H. MITCHELL,
AND W. B. NEVENS

*Division of Animal Nutrition and the Department of Dairy Husbandry,
University of Illinois, Urbana, Illinois*

Information available up to 1942 on the nutrition of the calf has been adequately reviewed by Savage and McCay (9). Johnson, Loosli, and Maynard (4) used purified diets to study the growth requirements of dairy calves. They reported growth rates which were below normal when compared to Ragsdale's standards (7). They stated that poor food consumption, associated with periodic digestive upsets, seemed to be largely responsible for the slow growth. Madsen, McCay, and Maynard (6) were successful in rearing sheep on synthetic rations for a period of 480 days after weaning, but obtained less satisfactory results with goats, guinea pigs, and rabbits.

In this paper we report the development of a synthetic diet which is satisfactory for the nutrition of the young dairy calf.

EXPERIMENTAL

Male dairy calves 24 to 48 hours old which had been allowed to receive colostrum were used as experimental animals. They were housed in individual metal cages 5×6 feet in size and equipped with heavy wire mesh bottoms. When the animals were received, they were given a capsule containing 100,000 I.U. of vitamin A and an injection of anti-scour serum. All diets were compounded to simulate milk. For the first week the calves were fed from pails equipped with rubber nipples, and at the end of this time they were taught to drink from the bucket. The animals were fed at a level of one pound of synthetic milk for each ten pounds of body weight. They were fed twice a day and given half the daily intake at each feeding. All vitamin supplements were given at the morning feeding. The synthetic milk was always fed at a temperature of $37°$ C.

The synthetic milk rations contained 13 per cent solids, similar to the solids content of cow's milk. The casein was dissolved according to the method of Bird et al (2). Sodium bicarbonate equal to 4.75 per cent of the weight of the casein was added to water at a temperature of 60° C. The casein was added slowly to the sodium bicarbonate solution and stirred with constant high speed (a "Lightnin" mixer was used[3]). When the casein

TABLE 1

Composition of synthetic milks

Components	Ration 1	Ration 2	Ration 3	Vitamins added[5]	Mg. per kg. liquid milk		
					Ration 1	Ration 2	Ration 3
	%	%	%				
Casein "Labco"	30.0	30.0	30.0	Thiamin	0.39	0.65	0.65
Soybean oil	12.0	....	....	Riboflavin	1.95	0.65	0.65
Lard	....	26.6	26.3	Pyridoxine	0.65	0.65	0.65
Salts 1[1]	6.0	6.0	....	Calcium panto-			
Salts 2[2]	....	....	4.0	thenate	1.95	1.30	1.30
Cerelose	47.0	37.4	39.4	Nicotinic acid	....	2.60	2.60
Wheat germ oil	....	....	0.3[4]	Ascorbic acid	....	13.0	....
Liver[3]	5.0	....	....	α-Tocopherol	....	1.0[6]	....
				2-Methyl-1,4- naphtho- quinone	....	0.26[6]	0.26[6]
				Inositol	....	....	26.0
				Choline	....	....	260.0
				p-Aminobenzoic acid	....	....	2.60
				Pteroyl-glutamic acid (folic acid)	....	....	0.052
				Biotin	....	....	0.01
				Vitamin A	5,000 I.U. per day		
				Vitamin D	500 I.U. per day		

[1] Salts 446 used in this laboratory, unpublished.

[2] Salt mixture of Phillips, P. H., and Hart, E. B., Jour. Biol. Chem., 109: 657 (1935), and modified by addition of cobalt chloride and increasing the manganese content. These salts were preferred because they dissolved more readily in the liquid rations.

[3] A defatted pork liver prepared and donated by the VioBin Corporation, Monticello, Illinois, through the courtesy of Mr. Ezra Levin.

[4] The 2-methyl-1,4-naphthoquinone, dissolved in the wheat germ oil, and the lard were homogenized into the solution of casein, salts, and cerelose.

[5] The water-soluble vitamins were made up in 25 per cent alcohol.

[6] The α-tocopherol and 2-methyl-1,4-naphthoquinone were dissolved in 95 per cent alcohol.

was in solution, cerelose was added. The salt mixture was dissolved in boiling water and then added to the casein-cerelose solution. After the entire mixture had been stirred for approximately one hour, the fat was homogenized into the mixture under a pressure of approximately 3,000 lbs. The synthetic milk was then pasteurized and stored in a refrigerator at a temperature of 5° C. until used. The synthetic milk thus prepared had a

[3] Manufactured by Mixing Equipment Co., Rochester, New York.

pH of 6.5 to 6.8. The compositions of the liquid rations used are given in table 1.

A capsule containing 5,000 I.U. vitamin A, 500 I.U. vitamin D, 50 mg. nicotinic acid, and 250 mg. ascorbic acid was given each animal daily.

Two animals, placed on ration 1, appeared to be doing well at the end of one week. During the second week both animals began to scour and appeared to be excreting a considerable amount of fatty material. The scours became progressively worse, and the animals refused to eat, lost weight, and presented a very unthrifty appearance. An attempt was made to save the animals by substituting a diet of cow's milk and treating scours by adminis-
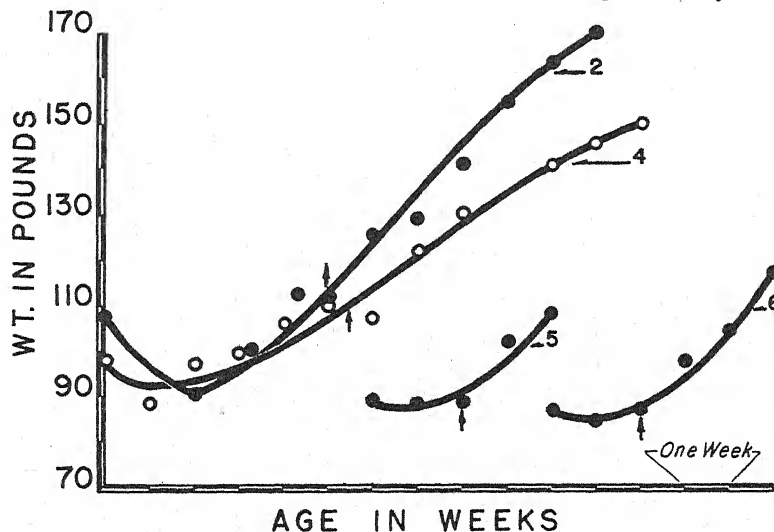
FIG. 1. Growth of calves on soybean oil–skim milk and lard–skim milk rations. Upward-pointing arrow indicates change from soybean oil–skim milk ration to lard–skim milk ration.

tration of sulfathalidine. Calf no. 1 became worse and died. Post-mortem examination showed the cause of death to be infectious scours. The condition of calf no. 2 improved on the diet of cow's milk and the animal finally recovered.

Gullickson, Fountaine, and Fitch (3) have reported that calves fed a diet of soybean oil homogenized into skim milk grew poorly, scoured, and appeared emaciated and unthrifty. Since soybean oil was included in ration 1, an attempt was made to determine whether this oil could be used satisfactorily in calf rations. Calves nos. 2, 3, 4, 5, and 6 were placed on a diet of skim milk into which soybean oil was homogenized at a level of 4 per cent on the liquid basis. After several days on this diet the animals started to scour, became very unthrifty in appearance and grew poorly. The volume of fecal material was large and appeared to contain large quantities

of undigested matter. The feces contained up to 30 per cent ether extract on the dry basis.

On the soybean oil–skim milk diet, calf no. 3 had severe scours, lost weight and became very weak. The animal, changed to a diet of whole cow's milk, still did not improve and was taken off the experiment. Autopsy showed the animal had gastro-enteritis.

Since the other animals (calves nos. 2, 4, 5, 6) were not doing well on this diet, it was decided to try some other fat. Lard was selected because the work of Gullickson *et al.* (3) had shown that calves fed a diet of lard homogenized into skim milk made good gains in weight, remained in a healthy and thrifty condition, and exhibited only occasional scours. The animals were changed to a diet of skim milk into which lard was homogenized at a
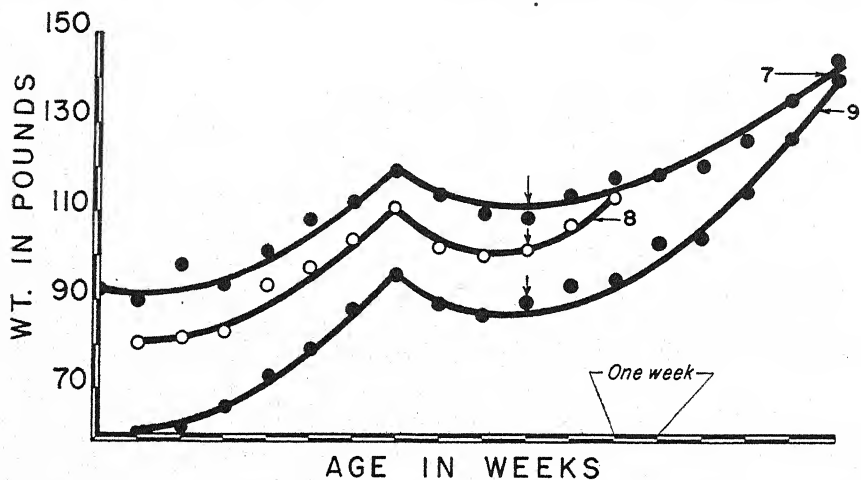
FIG. 2. Growth of calves on rations 2, 4, and 5. Break in curve at 7 weeks occurred with change to ration 4. Downward-pointing arrow indicates change to ration 5.

level of 4 per cent on the liquid basis. After several days on this diet the animals stopped scouring, became healthy and thrifty in appearance, and made gains in weight. The growth data are summarized in figure 1.

From these results it was apparent that the type of fat used in the diet of the dairy calf is of importance. Ration 2 was prepared and homogenized with water, as previously described.

Three animals, calves nos. 7, 8, and 9, were placed on ration 2. They made good gains in weight, appeared healthy and thrifty, and scoured infrequently. The growth data are given in figure 2.

After 6 weeks on the liquid diet, a change to a solid diet was attempted. The animals would not eat the dry ration except when fed as a slurry. The composition of ration 4 is given in table 2. On this ration the animals scoured and lost weight. Decreasing the lard content to 4 per cent, chang-

ing the salt mixture, or adding roughage (wood flock) did not prevent scours. Even the addition of *p*-aminobenzoic acid, inositol, choline, pteroyl-glutamic acid ("folic acid") and biotin did not improve the condition of the animals. Only after the substitution of starch for some of the cerelose did the animals stop scouring and gain weight. The composition of ration 5, on which the calves showed good growth, appeared healthy and thrifty, and did not scour, is given in table 2.

Rations 1 and 2, previously described, did not give completely satisfactory results for rearing young calves. The vitamin supplement was modified to contain all the pure crystalline compounds that have been re-

TABLE 2

Composition of dry rations

Components	Ration 4	Ration 5	Vitamins added	Mg. per kg.	
				Ration 4	Ration 5
	%	%			
Casein "Labco"	20.0	20.0	Thiamin	5.0	5.0
Lard	8.0	4.0	Riboflavin	5.0	5.0
Salts 1	6.0	....	Nicotinic acid	20.0	20.0
Salts 2	....	4.0	Pyridoxine	5.0	5.0
Magnesium carbonate	0.7	....	Calcium pantothenate	10.0	10.0
Cerelose	65.3	20.0	Ascorbic acid	100.0	....
Methionine	....	0.3	*p*-Aminobenzoic acid	....	20.0
Wheat germ oil	....	0.3	Inositol	....	200.0
Wood flock	....	10.0	Choline	....	2,000.0
Starch	....	41.4	Pteroyl-glutamic acid (folic acid)	....	0.4
			Biotin	....	0.1
			2-Methyl-1,4-naphtho-quinone	....	2.0
			Vitamin A	5,000 I.U. per day	
			Vitamin D	500 I.U. per day	

ported to be members of the vitamin B complex. Two animals have been raised on ration 3 for a period of 12 weeks. The growth of the calves was normal as compared to Ragsdale's standards (7), and they were healthy and thrifty in appearance. The scours occurring on two or three occasions were cured by administration of sulfathalidine. The growth data are given in figure 3.

Ascorbic acid was not included in the vitamin supplement of ration 3 as it was found unnecessary for the young calf. Several calves have been raised without vitamin C in the diet and showed normal growth, appeared healthy and thrifty, and did not scour. In addition, they did not develop navel ill, which Lundquist and Phillips (5) reported to be caused by a vitamin C deficiency. Blood ascorbic acid levels have been determined by the method of Roe and Keuther (8) and are given in table 3. The values are normal by comparison to those reported by Lundquist and Phillips (5), who found the ascorbic acid content of calf blood varied between 0.1 and 0.8 mg. per 100 cc. of whole blood.

TABLE 3

Ascorbic acid levels of calves' whole blood

Calves on diet containing ascorbic acid								Calves on ascorbic acid–free diet									
Calf 7		Calf 8		Calf 9		Calf 10		Calf 11		Calf 12		Calf 13		Calf 14		Calf 15	
Age in days	Mg./100 cc.	Age in days	Mg./100 cc.	Age in days	Mg./100 cc.	Age in days	Mg./100 cc.	Age in days	Mg./100 cc.	Age in days	Mg./100 cc.	Age in days	Mg./100 cc.	Age in days	Mg./100 cc.	Age in days	Mg./100 cc.
45	0.25	30	0.42	30	0.82	7	0.75	2	0.81	2	0.60	2	0.94	2	1.04	2	0.72
52	0.50	37	0.94	37	0.66	14	0.77	9	0.29	9	0.47	8	0.80	8	0.63		
59	0.51	44	0.83	44	0.70	21	0.90	16	0.53	16	0.55	23	0.39	23	0.38		
73	0.46			79	0.59	28	0.93	23	0.58	23	0.76						
								30	0.77	30	0.47						
								37	0.49	37	0.51						
								44	0.66	44	0.84						
										51	0.57						

Ascorbic acid was fed to calf no. 7 until 70 days of age and to calves nos. 8 and 9 until 56 days of age.   After this time these animals received an ascorbic acid–free diet.   Calves nos. 10, 11, 12, 13, 14, and 15 did not receive any ascorbic acid during the course of the experiment.   The data in table 3 show that the blood level of ascorbic acid does not decrease to any great extent when the animal is fed an ascorbic acid–free diet.   This indicates that the calf can synthesize vitamin C in its body tissues and does not need it in the diet.
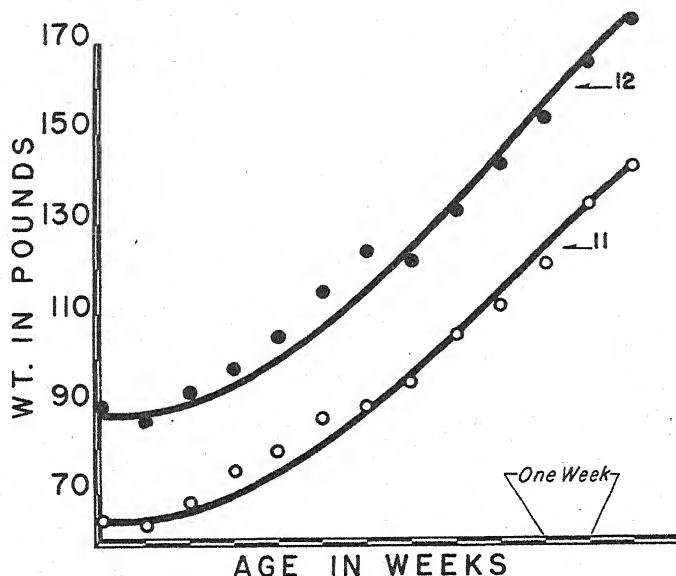
FIG. 3.   Growth of calves on ration 3.

DISCUSSION

A synthetic milk diet (ration 3) has been developed for studies of the nutrition of the young dairy calf.   Day-old calves have been raised on this ration for a period of 12 weeks.   They have shown good growth, were healthy and thrifty in appearance, and seldom scoured.

When the ration was fed in the dry form, less satisfactory results were obtained.   It may be that the physical characteristics of this diet or the method of feeding (as a slurry) caused the unsatisfactory results.   Bate, Espe, and Cannon (1) have reported that dairy calves did poorly on a diet of skim milk and unhomogenized fat.   Calves fed the same diet but with the fat homogenized into the skim milk did better.   Since rations 4 and 5 were fed as slurries, the fat was unhomogenized.   This may have been the cause of the poor results obtained.

The data presented in table 3 on the blood levels of ascorbic acid indicate that the young dairy calf does not require ascorbic acid.   Lundquist and Phillips (5) have reported that the young dairy calf requires ascorbic acid

only if the animal is receiving an insufficient amount of vitamin A. The animals in the experiments reported in this paper all received 5,000 I.U. of vitamin A per day. This may account for the fact that the results obtained do not show a great decrease in the blood level of ascorbic acid.

SUMMARY

1. A synthetic milk has been developed for the young dairy calf. This ration will support good growth over a period of 12 weeks from birth. The animals raised on this diet were healthy and thrifty in appearance.

2. On rations containing liberal amounts of vitamin A, the young dairy calf does not require vitamin C.

3. Calves that received soybean oil in the diet grew poorly, scoured, and appeared unthrifty, whereas the animals that received the rations prepared with lard showed good gains in weight, did not scour, and appeared normal and healthy.

4. The calves did much better in all respects when fed the synthetic milk diet than when solid diets fed as a slurry were used.

REFERENCES

(1) BATE, W., ESPE, D., AND CANNON, C. Y. Influence of Homogenization of Fat on Haircoat of Dairy Calves. Jour. Dairy Sci., 29: 41–43. 1946.

(2) BIRD, E. W., SADLER, H. W., AND IVERSON, C. A. The Preparation of a Non-Desiccated Sodium Caseinate Sol and Its Use in Ice Cream. Iowa Agr. Expt. Sta. Res. Bul. 187: 177–208. 1935.

(3) GULLICKSON, T. W., FOUNTAINE, F. C., AND FITCH, J. B. Various Oils and Fats as Substitutes for Butterfat in the Ration of Young Calves. Jour. Dairy Sci., 25: 117–128. 1942.

(4) JOHNSON, P. E., LOOSLI, J. K., AND MAYNARD, L. A. Purified Diet Studies with Calves. Jour. Dairy Sci., 23: 553–554. 1940.

(5) LUNDQUIST, N. S., AND PHILLIPS, P. H. Certain Dietary Factors Essential for the Growing Calf. Jour. Dairy Sci., 26: 1023–1030. 1943.

(6) MADSEN, L. L., McCAY, C. M., MAYNARD, L. A., DAVIS, G. K., AND WOODWARD, J. C. Synthetic Diets for Herbivora, with Special Reference to the Toxicity of Cod-Liver Oil. N. Y. (Cornell) Agr. Expt. Sta. Mem. 178. 1935.

(7) RAGSDALE, A. C. Growth Standards for Dairy Cattle. Mo. Agr. Expt. Sta. Bul. 336. 1934.

(8) ROE, J. H., AND KEUTHER, C. A. The Determination of Ascorbic Acid in Whole Blood and Urine through the 2,4,-Dinitrophenylhydrazine Derivative of Dehydroascorbic Acid. Jour. Biol. Chem., 147: 399–407. 1943.

(9) SAVAGE, E. S., AND McCAY, C. M. The Nutrition of Calves; A Review. Jour. Dairy Sci., 25: 595–650. 1942.

A SUGGESTED MODIFIED BABCOCK PROCEDURE FOR TESTING HOMOGENIZED MILK[1]

P. S. LUCAS AND G. M. TROUT

Michigan Agricultural Experiment Station, East Lansing, Michigan

With increasing consumer acceptance of homogenized milk, considerable interest has been shown in the testing of such milk for fat either by the standard Babcock method or by a modification. In general, early work and statements conflicted relative to the accuracy of the Babcock test for homogenized milk. However, there was general agreement that the homogenized milk tested within the 0.1 per cent tolerance of the Babcock procedure. Some accepted the theory that the fat globules, being reduced in size by the process, could not be centrifuged sufficiently to give a test comparable to that of nonhomogenized milk; consequently, the Babcock fat test of homogenized milk could be expected to be not more than 0.1 per cent lower than that of nonhomogenized milk.

If a slightly lower test value were the only factor involved in making a Babcock fat test of homogenized milk, likely the regular procedure would be accepted and used in routine work. However, workers soon recognized that char formation was greater in applying the test to homogenized than to nonhomogenized milk. This char often gave the appearance of a burned test. If less acid were used, the test often appeared curdy. This char or curd usually appeared as a thin disc or plug at the base of the fat column. When the char was reduced to a minimum, its presence did not affect appreciably the reading of the fat column. In routine testing, however, the presence and appearance of this char in greater or lesser amounts, intermixed with or at the base of the fat column, often led one to question the accuracy of the test. Consequently, many modifications of the Babcock procedure, in part with the aim of preventing char formation and thus increasing the accuracy of the test, have been recommended for testing homogenized milk.

The literature on this subject has been reviewed, in part, in a previous paper (9), which showed that at least 12 modifications of the standard Babcock procedure have been recommended for homogenized milk. Variants involved in the different modifications included: (a) strength of the sulphuric acid used, (b) amount of acid used, (c) temperature of the acid, (d) addition of acid ranging from three to five portions, (e) temperature of the milk, (f) prolonged mixing of acid and milk, (g) remixing acid-water-serum mixture after centrifuging, and (h) prolonged centrifuging.

EXPERIMENTAL METHODS

In comparing the various modifications recommended, it became evident that the procedures involving prolonged action of the sulphuric acid at a relatively high concentration without burning the test offered the best solution to the problem. This, in effect, entailed the tempering of the milk and acid to 70° F., the use of a relatively large portion of the acid (1.83 sp. gr. sulphuric acid) at the first addition, thorough mixing of the acid and milk after each addition, and prolonged agitation of the milk-acid mixture after all the acid was added. As a result of these observations and after comparing the numerous modifications of the test, the following suggested modified Babcock procedure for testing homogenized milk was adopted:

(a) Temper the acid and milk to 70° F.

(b) Use sulphuric acid of 1.83 to 1.835 sp. gr.

(c) Use the full amount of sulphuric acid (17.5 ml.).

(d) Add the acid in three portions, 8, 5, and 4.5 ml., respectively.

(e) Mix the acid and milk by rotary motion after each addition and continue agitation for at least 15 seconds before adding the second and third increments of sulphuric acid.

(f) Shake the tests in a mechanical shaker for at least 2 minutes before centrifuging.

(g) Centrifuge and add hot water in accordance with the regular Babcock procedure. Substitution of a water-alcohol (ratio 1.4:1 by weight) solution for the hot water to bring the fat up into the neck of the bottle for reading is optional.

This procedure was followed carefully in testing in duplicate a series of 36 milk samples, both nonhomogenized and homogenized. These samples also were tested in duplicate by the Mojonnier method, which was used as a standard for accuracy. However, instead of using a volumetrically measured approximately 10-gram portion, the samples previously tempered to 70° F. were weighed carefully on a chemical balance directly into fat-extraction flasks.

The nonhomogenized samples were taken from the vat after pasteurization prior to homogenization. The milk had been kept thoroughly mixed during pasteurization and homogenization in order to insure uniform fat distribution. The homogenized samples were taken from the cooled, bottled product after homogenization was well under way.

Homogenization was accomplished by means of a 500-gallon-per-hour viscolizer at 2500 pounds pressure at 130° F. following pasteurization. All the 17.5-ml. portions of milk were pipetted into the calibrated test bottles at one time to assure correct sampling after the milk had been tempered at 70° F. for two hours.

In studying the accuracy of the suggested modified Babcock procedure, the procedure was tried on the same milk unhomogenized and homogenized.

Percentages of fat in nonhomogenized and homogenized milk as determined by the Mojonnier method

| Trial no. | Date | Mojonnier fat test on milk which was | | | | | | Variation of average fat test of homogenized milk from that of nonhomog. milk |
| | | Not homogenized | | | Homogenized | | | |
		Duplicates	Difference between duplicates	Average test	Duplicates	Difference between duplicates	Average test	
		%		%	%		%	
1	12-13-44	3.735 3.698	0.037	3.713	3.705 3.722	0.017	3.714	+ 0.001
2	12-14-44	3.928 3.933	0.005	3.930	3.962 3.943	0.019	3.952	+ 0.022
3	12-15-44	4.024 4.047	0.023	4.035	4.058 4.073	0.015	4.064	+ 0.029
4	12-18-44	3.973 3.957	0.016	3.965	3.976 3.976	0.000	3.976	+ 0.011
5	12-19-44	3.918 3.867	0.051	3.892	3.888 3.903	0.015	3.895	+ 0.003
6	12-20-44	3.984 4.033	0.049	4.008	4.064		4.064	+ 0.056
7	12-21-44	4.068		4.068	3.994 3.948	0.046	3.971	− 0.097
8	1-15-45	3.966 3.906	0.060	3.936	3.931 3.888	0.043	3.910	− 0.026
9	1-16-45	3.702		3.702	3.706		3.706	+ 0.004
10	1-17-45	3.898 3.899	0.001	3.898	3.876 3.887	0.011	3.881	− 0.017
11	1-18-45	3.848 3.849	0.005	3.848	3.847 3.868	0.021	3.857	+ 0.009
12	1-19-45	3.980 3.973	0.007	3.977	3.982 3.996	0.014	3.989	+ 0.012
13	1-22-45	5.012 5.038	0.026	5.025	5.049 5.040	0.009	5.044	+ 0.019
14	1-23-45	4.956 4.973	0.017	4.964	4.936 4.934	0.002	4.935	− 0.029
15	1-24-45	4.575 4.628	0.053	4.601	4.653 4.634	0.019	4.643	+ 0.042
16	1-25-45	5.124 5.082	0.042	5.103	5.047 5.082	0.035	5.064	− 0.039
17	1-26-45	4.470 4.476	0.006	4.473	4.493 4.503	0.010	4.498	+ 0.025
18	1-29-45	4.591 4.589	0.002	4.590	4.580 4.600	0.020	4.590	0.000
19	1-30-45	4.763 4.761	0.002	4.762	4.759 4.768	0.009	4.763	+ 0.001
20	1-31-45	4.619 4.606	0.013	4.612	4.608 4.606	0.002	4.607	− 0.005
21	2-1-45	4.532 4.537	0.005	4.534	4.543 4.552	0.009	4.547	+ 0.013
22	2-2-45	4.585 4.575	0.010	4.580	4.560 4.570	0.010	4.565	− 0.015
23	2-5-45	4.375 4.409	0.034	4.392	4.407 4.404	0.003	4.405	+ 0.013
24	2-6-45	4.606 4.627	0.021	4.616	4.641 4.644	0.003	4.642	+ 0.026
25	2-7-45	4.591 4.596	0.005	4.593	4.597 4.586	0.011	4.591	− 0.002
26	2-8-45	4.786 4.789	0.003	4.789	4.785 4.777	0.008	4.781	− 0.006
27	2-9-45	4.556 4.592	0.036	4.574	4.594 4.593	0.001	4.593	+ 0.019
28	2-19-45	3.831 3.804	0.027	3.818	3.804 3.815	0.011	3.810	− 0.008
29	2-20-45	3.708 3.699	0.009	3.704	3.701 3.694	0.007	3.698	− 0.006
30	2-22-45	3.651 3.650	0.001	3.650	3.672 3.671	0.001	3.671	+ 0.021
31	2-23-45	3.897 3.926	0.029	3.912	3.918 3.903	0.015	3.911	− 0.001
32	2-26-45	3.723 3.716	0.007	3.720	3.724 3.738	0.014	3.731	+ 0.011
33	2-27-45	3.695 3.716	0.021	3.706	3.712 3.695	0.017	3.704	− 0.002
34	2-28-45	3.850 3.864	0.014	3.857	3.835 3.832	0.003	3.834	− 0.023
35	3-1-45	3.814 3.801	0.013	3.807	3.826 3.817	0.009	3.821	+ 0.014
36	3-2-45	3.916 3.886	0.030	3.901	3.898 3.882	0.016	3.890	− 0.011
Averages		(Arithmetic) ...	0.0189	4.2015	....	0.0124	4.2032	0.0177
		(Algebraic) ...			....			+ 0.0017

Thus, comparisons are made on homogenized milk tested under the same conditions as the nonhomogenized samples. In other words, the regular Bab-

TABLE 2

Comparison of modified Babcock and Mojonnier tests on homogenized milk

Trial no.	Date	Modified Babcock test of milk			Variation of modified Babcock test from Mojonnier test	
		Not homo-genized	Homo-genized		Not homo-genized	Homo-genized
		Average of duplicates	Average of duplicates	Difference		
		%	%			
1	12–13–44	3.725	3.70	− 0.025	+ 0.008	− 0.014
2	12–14–44	4.00	4.00	0.00	+ 0.070	+ 0.048
3	12–15–44	4.10	4.10	0.00	+ 0.065	+ 0.036
4	12–18–44	4.10	4.10	0.00	+ 0.135	+ 0.124
5	12–19–44	3.95	3.95	0.00	+ 0.058	+ 0.055
6	12–20–44	4.10	4.05	− 0.05	+ 0.092	+ 0.014
7	12–21–44	4.05	4.025	− 0.025	− 0.018	+ 0.054
8	1–15–45	4.00	4.00	0.00	+ 0.064	+ 0.090
9	1–16–45	3.80	3.775	− 0.025	+ 0.098	+ 0.069
10	1–17–45	3.925	3.90	− 0.025	+ 0.027	+ 0.019
11	1–18–45	3.90	3.90	0.00	+ 0.052	+ 0.043
12	1–19–45	4.025	4.00	− 0.025	+ 0.048	+ 0.011
13	1–22–45	5.075	5.125	+ 0.05	+ 0.050	+ 0.081
14	1–23–45	5.00	4.95	− 0.05	+ 0.036	+ 0.015
15	1–24–45	4.675	4.65	− 0.025	+ 0.074	+ 0.013
16	1–25–45	5.175	5.15	− 0.025	+ 0.072	+ 0.086
17	1–26–45	4.525	4.525	0.00	+ 0.052	+ 0.027
18	1–29–45	4.65	4.625	− 0.025	+ 0.060	+ 0.035
19	1–30–45	4.80	4.80	0.00	+ 0.038	+ 0.037
20	1–31–45	4.70	4.70	0.00	+ 0.088	+ 0.093
21	2– 1–45	4.575	4.575	0.00	+ 0.041	+ 0.028
22	2– 2–45	4.625	4.625	0.00	+ 0.045	+ 0.060
23	2– 5–45	4.475	4.475	0.00	+ 0.033	+ 0.070
24	2– 6–45	4.675	4.70	+ 0.025	+ 0.059	+ 0.058
25	2– 7–45	4.625	4.625	0.00	+ 0.032	+ 0.034
26	2– 8–45	4.80	4.80	0.00	+ 0.013	+ 0.019
27	2– 9–45	4.675	4.60	− 0.075	+ 0.101	+ 0.007
28	2–19–45	3.85	3.85	0.00	+ 0.032	+ 0.040
29	2–20–45	3.725	3.70	− 0.025	+ 0.021	+ 0.002
30	2–22–45	3.725	3.70	− 0.025	+ 0.075	+ 0.089
31	2–23–45	3.95	3.925	− 0.025	+ 0.038	+ 0.014
32	2–26–45	3.775	3.775	0.00	+ 0.055	+ 0.044
33	2–27–45	3.775	3.75	− 0.025	+ 0.069	+ 0.046
34	2–28–45	3.90	3.875	− 0.025	+ 0.043	+ 0.041
35	3– 1–45	3.90	3.875	− 0.025	+ 0.093	+ 0.054
36	3– 2–45	3.975	3.925	− 0.05	+ 0.074	+ 0.035
Averages (Arithmetic)		4.258	4.244	0.018	0.058	0.045
(Algebraic)		....	....	− 0.014	+ 0.057	+ 0.044

cock procedure was not used as a standard for the nonhomogenized milk. It was believed that no modification of the Babcock procedure would improve the accuracy of the regular Babcock procedure on nonhomogenized

milk. Consequently, the modified Babcock technique was used on the non-homogenized milk also, and the results accepted as though they were obtained by the regular procedure. A few trials run on nonhomogenized milk by the regular and modified procedures showed the tests to be comparable in every respect.

RESULTS

The data are presented in tables 1 and 2. The average tests of the 36 nonhomogenized and homogenized samples, as determined by the Mojonnier method, were practically identical; the homogenized milk averaged 0.0017 per cent higher than the same milk not homogenized. A study of the duplicate Mojonnier tests showed less variation, on the average, between the tests of homogenized milk than between those of the nonhomogenized milk, although there were several exceptions.

The modified Babcock method yielded tests on homogenized milk on the average within 0.018 per cent of that of the same milk not homogenized. Of the 36 tests, 16 (or 44 per cent) checked with those of the nonhomogenized milk; 15 (or 41 per cent) were 0.025 per cent lower; one was 0.025 per cent higher; two were 0.05 per cent lower; one was 0.05 per cent higher; and one was 0.075 per cent lower. The average algebraic difference was −0.014 per cent lower than that of the nonhomogenized milk.

DISCUSSION

In presenting the suggested modified Babcock procedure for testing homogenized milk, no claim is made for originality. The suggested technique resulted from observations made while testing homogenized milk by the many recommended procedures, results of which already have been published (9). Particular use was made of the principles involved in some of the methods (1, 5, 6, 7, 8, 10). During this earlier study (9) it appeared necessary to prolong action of the sulphur acid on the milk at a concentration to give maximum digestion without burning. Also, continued agitation of the acid-milk mixture following final addition of the acid seemed to facilitate more complete digestion of the caseous matter. These modifications, while involving more time than the regular Babcock method, did not require as much time as did some of the suggested methods. Considerable importance is associated with the correct temperature, both of the acid and of the milk. The procedure used was based on temperatures of 70° F. With this temperature a relatively large volume of sulphuric acid could be introduced into the milk at the first addition without harmful results. Maintaining maximum chemical reaction through further additions of acid and prolonged agitation before centrifuging seem imperative.

Also, importance is associated with the addition of the full volume (17.5 ml.) of sulphuric acid rather than reducing the total volume. This seems to be in accordance with the work of Bailey (3), who showed in his extensive

studies on the Babcock test that casein was digested with more difficulty when fat was homogenized into the solution. Data indicated that, as the volume of acid was increased, a lesser percentage of insoluble organic material remained in the mixture.

The feature of remixing the acid-milk mixture after either the first or second centrifugings, and before or after the addition of all or a part of the water (1, 5, 6, 7, 10), was not incorporated into the suggested technique. Such remixing would seem to indicate that the caseous matter was not wholly digested at the time of centrifuging. Apparently if the original mixing and agitation under the best conditions of temperature and concentration of acid were insufficient to digest all the adsorbed casein, then lower temperatures, which naturally follow during the carrying out of the procedure, and a diluted acid, due to the addition of water, would not facilitate further digestion. Furthermore, by remixing the centrifuged fat with the acid-serum-water-liquid, a partial loss of the benefits of the preceding centrifuging would seem to occur. Retention of the caseous matter in its digested form through the addition of hot water (140° F. or above) or other liquids (2, 3) in bringing the liberated fat up into the neck of the bottle for reading seems important. Beautifully clear fat columns were obtained when a hot sulphuric acid–water mixture (7:10) was added instead of water after the centrifugings to bring the fat up for reading (2). However, one lot of 12 tests made using this variant averaged 0.18 per cent lower on the homogenized milk than on the nonhomogenized milk; in another series of 15 samples, the difference was 0.12 per cent lower. Hence, the introduction of an acid-water mixture instead of hot water to raise the fat offered no possibilities.

Brueckner's (4) recommendation of adding a water-alcohol mixture (ratio 1.4:1 by weight) to the tests of homogenized milk to bring the fat up into the column before final centrifuging appears to have considerable merit. Despite any char formation, the tendency of which seems to be reduced, the fat column is clear, is supported by a clear solution, and has well-defined menisci. However, the results of a few trials indicate that the fat-test reading is slightly higher when the water-alcohol solution is used to support the fat column of homogenized milk than when water alone was used. This slightly increased reading may offset, in part, the slightly lower test generally reported on Babcock tests of homogenized milk. Several factors, such as the slight solubility of alcohol in fat and *vice versa*, may contribute to the slightly higher reading of the fat column of homogenized milk when using the water-alcohol mixture to force up the fat, but the appearance of the fat column with its relatively deep menisci supported by a perfectly clear liquid would indicate that such fat columns were in the best condition for reading. The menisci were well defined, probably due to the drying and cleaning action of the alcohol on the glass, thereby enhancing the capillarity of the fat. Thus, the seemingly increased depth of menisci might

be sufficient to account for the slightly higher reading. Reports of use of the water-alcohol solution to bring up the fat column on Babcock tests of homogenized milk in routine testing in a commercial laboratory indicate much satisfaction over its use.

In view of the many modifications of the Babcock method for testing homogenized milk, the authors are extremely reluctant to suggest another technique. However, in the interest of clarifying and unifying the procedure with a minimum of time involved, the apparently most satisfactory features of the various methods were incorporated into one method. It is hoped that this procedure will simplify and lend greater accuracy to the Babcock testing of homogenized milk, rather than add to the confusion.

SUMMARY

A modified Babcock fat test for homogenized milk, employing the best features of the many modifications now recommended, has been suggested. The technique involves tempering the milk and acid to 70° F.; using at least 17.5 ml. of sulphuric acid with sp. gr. of 1.83 to 1.835; adding the acid in three portions, the first consisting of approximately one half of the acid; and prolonging the agitation of the milk-acid mixture prior to centrifuging. The addition of a water-alcohol mixture (ratio 1.4:1 by weight) to the test instead of water alone before final centrifuging adds to the clarity of the fat column without appreciably affecting the reading.

Employing the suggested technique, the arithmetic-average fat test of 36 samples of homogenized milk was 0.018 per cent lower than that of the same test applied to similar milk not homogenized. The arithmetic-average variations of the 36 tests from the Mojonnier tests were + 0.058 and + 0.045 per cent for nonhomogenized and homogenized milk, respectively.

Credit is due Mr. Robert Frantz for the making of the tests reported herein.

REFERENCES

(1) Anonymous. Modified Babcock Test for Homogenized Milk. (Mimeo.)

(2) BABCOCK, S. M. A New Method for the Estimation of Fat in Milk, Especially Adapted to Creameries and Cheese Factories. Wisc. Agr. Expt. Sta. Bul. 24. 1890.

(3) BAILEY, D. E. Study of Babcock Test for Butterfat in Milk. Jour. Dairy Sci., 2: 331–373. 1919.

(4) BRUECKNER, H. J. Babcock Test for Homogenized Milk and Cream. Amer. Milk Rev., 2(6): 139. 1940.

(5) DOAN, F. J. Laboratory Control of Homogenized Milk. Internatl. Assoc. Milk Dealers, Assoc. Bul. 23. 1943.

(6) DOAN, F. J., JOSEPHSON, D. V., AND ADAMS, J. Stability of the Fat Emulsion of Homogenized Milk. Milk Dealer, 31(2): 35, 54, 58–61. 1941.

(7) MARQUARDT, J. C. A Method for Determining Fat in Homogenized Milk. Milk Dealer, 31(5): 24. 1942.

(8) TRACY, P. H.   Problems in the Processing and Marketing of Homogenized Milk.
     N. Y. State Assoc. Dairy and Milk Insp. Ann. Rpt., 12: 69–84.   1938.

(9) TROUT, G. M., AND LUCAS, P. S.   A Comparison of Various Modifications of the
     Babcock Test for the Testing of Homogenized Milk.   Jour. Dairy Sci., 28: 901–
     919.   1945.

(10) WHITAKER, R.   Recommendations for the Standardization of the Method of Deter-
     mining the U.S.P.H.S. Index of Homogenization of Milk.   Internatl. Assoc. Milk
     Dealers, Assoc. Bul. 6.   1944.

METHODS OF PRESERVING GRASS SILAGE AND VITAMIN A POTENCY OF MILK PRODUCED THEREFROM[1]

J. J. STEFANIAK, I. W. RUPEL,[2] G. BOHSTEDT AND W. H. PETERSON

Departments of Biochemistry and Dairy Husbandry, College of Agriculture,
University of Wisconsin, Madison, Wisconsin

In previous publications a number of methods for the preservation of grass forages have been reported (3, 7). The investigation has been continued with several forages to which were added various materials to aid in preservation. In addition to chemical analysis of the forages and silages, short-time feeding tests have been made on all the silages to determine their palatability, and longer feeding trials have been made on the most important lots in order to obtain data regarding milk production and the vitamin A potency of the milk produced by cows fed these silages. The work has been extended to several farms which were part of an experimental grass silage program sponsored by the College of Agriculture.

EXPERIMENTAL

Silages. In preliminary experiments, forages treated in different ways were ensiled in layers in silos 8 or 10 feet in diameter. Each layer contained one to 5 tons of forage. The preservative was added to the forage as it was passed through the silage cutter in order to obtain uniform distribution. When the silo was opened, the silages were analyzed and fed, and the readiness with which the cows consumed each lot was recorded. The methods of preservation which had been most successful in the layer trials were further tested on a larger scale. Quantities of fresh forage ranging from 5 to 20 tons were ensiled and the resulting silages were used for feeding experiments. Chemical analyses of the forage and silage were made. Dry matter was obtained by drying in an electric oven at 105° C., and the pH of the silage was determined on the expressed juice by means of a glass electrode. The carotene content was determined by the method of Hegsted, Porter, and Peterson (2), except that a photoelectric colorimeter and a calibration curve of β-carotene in Skelly solve were used instead of a spectrophotometer. Light absorption by the carotene solution was measured with a 440-μ filter.

Milks. The silages used in the feeding trials were fed to lots of from five to nine cows each. The animals composing each lot were selected so that lactation, weight, and milk and butterfat production were approximately equal at the time the feeding trials were begun. Representative samples of morning and evening milkings were obtained from each lot of animals and

analyzed for their butterfat, carotene, and vitamin A content.   The carotene
and vitamin A contents of these milks were determined by the methods of
Olson, Hegsted, and Peterson (4) and Berl and Peterson (1).   The method
used for the extraction and saponification of the butterfat in the milk was
that of Olson *et al.*   The procedures used for drying the ethereal extract,
analysis for β-carotene in Skelly solve and vitamin A in chloroform were
those of Berl and Peterson (1).

RESULTS

Layer silages.   Table 1 summarizes the data for the layer silage experi-
ments.   In silo I, 9 different materials or treatments were tested to deter-
mine their usefulness as a means of preservation.   Acidity, which is usually
a good index of quality, ranged from pH 4.2 to 5.2.   The best layers from the
standpoint of palatability were those approximating 4.5 or less.   Silages
which have a pH above 4.5 often contain butyric acid, ammonia, and other
fermentation products having an objectionable odor.   The layers of alfalfa
preserved with Silogerm (a commercial bacterial culture for the preservation
of silage) and salt (I–2), and salt alone (I–4) had undergone a butyric type
of fermentation and were of poor quality.   Carotene preservation in these
two silages was reasonably good, however.   A butyric fermentation and
carotene preservation not uncommonly go together.   Butyric fermentation
occurs under anaerobic conditions, and exclusion of air favors carotene pres-
ervation.   The wilted alfalfa and the alfalfa ensiled without a preservative
were well preserved and palatable, showing that at times good silage may
be obtained without any additions.   However, these layers were far down
in the silo, where there was considerable pressure from above, and this con-
dition may have favored preservation.   The most palatable silages were pro-
duced by addition of corn meal, molasses, or concentrated soured whey to
the forage.   Concentrated soured whey was produced from whey which had
been inoculated with a 1 per cent inoculum of *Lactobacillus bulgaricus* and
incubated under anaerobic conditions at 110° F. for 5 days.   The soured
whey was concentrated in vacuo to one-seventh of the original volume and
applied to the forage at a rate of 70 pounds per ton.   The layers preserved
with concentrated soured whey and whey alone were very soggy.   There was
no correlation between carotene and pH or between carotene and palatability.

The differences between the dry-matter content of the forage and the
corresponding silage noted in tables 1 and 2 may be due, in part, to vari-
ations in sampling, but in some cases are due to additions either of liquid
(*e.g.*, whey) or of dry material (*e.g.*, dry sorghum fodder) to the forage at
the time of ensiling.   There also would be a tendency for the moisture con-
tent of the layers to become equal because of the movement of liquid or water
vapor from one layer to the next.

In 1941, the unavailability of molasses led to further experiments (layers
II–1 to II–9) in search of a suitable method for preserving grass silage by

TABLE 1

Composition of layer silages

Silo and layer[a]	Year	Forage	Addition or treatment	Preservative (lbs./ton)	Dry matter — Fresh forage (%)	Dry matter — Silage (%)	pH	Carotene (dry basis) — Fresh forage (μg./gm.)	Carotene (dry basis) — Silage (μg./gm.)	Palatability[e]
I-1	1940	Clover-timothy	Corn meal	150	36.2	36.5	4.3	130	86	Good
I-2	1940	Alfalfa	Silogerm and salt	b	28.1	22.1	5.2	192	118	Poor
I-3	1940	Alfalfa	Dry sorghum fodder	250	27.3	26.0	4.5	164	65	Good
I-4	1940	Alfalfa	Salt	10	27.3	23.5	5.2	164	142	Poor
I-5	1940	Alfalfa	None	None	27.3	25.4	4.6	164	83	Good
I-6	1940	Alfalfa	Wilted, 37% D.M.	None	48.6	35.3	4.8	92	70	Good
I-7	1940	Alfalfa	Wilted, 37% D.M., molasses	60	37.8	35.9	4.3	145	81	Excellent
I-8	1940	Alfalfa	Conc. soured whey	70	26.9	25.0	4.3	146	73	Good
I-9	1941	Alfalfa	Wilted, whey	600	42.2	32.5	4.2	100	80	Fair
II-1	1941	Sweet clover (3)-corn (1)	None	None	29.3	30.5	4.1	91	86	Good
II-2	1941	Sweet clover (1)-corn (1)	None	None	28.0	24.5	3.8	83	70	Good
II-3	1941	Alfalfa	None	None	22.9	22.8	4.7	172	5	Poor
II-4	1941	Alfalfa	Silogerm and salt	b	22.9	23.0	5.2	172	34	Poor
II-5	1941	Alfalfa	Vacatone	30	21.9	22.1	5.1	172	28	Good
II-6	1941	Alfalfa	Vacatone	60	21.9	24.6	5.0	172	47	Good
II-7	1941	Alfalfa	Wilted, 40% D.M.	None	43.3	25.6	4.9	72	87	Fair
II-8	1941	Alfalfa	Ground barley	200	31.8	31.7	4.4	172	117	Fair
II-9	1942	Alfalfa	Corn and cob meal	250	31.8	30.2	4.0	172	151	Excellent
III-1	1942	Alfalfa	Ground shelled corn	200	41.0	31.9	4.8	75	31	Good
III-2	1942	Alfalfa	Whole shelled corn	200	41.0	30.2	4.5	75	46	Good
III-3	1942	Alfalfa	Ground wood shavings	200		33.7	4.6		39	Poor
III-4	1942	Alfalfa	Oat straw	200	34.5	38.0	4.7	133	30	Poor
III-5	1942	Alfalfa	Dry sorghum fodder	200	29.9	43.7	4.5	140	29	Poor
III-6	1942	Alfalfa	Wilted, 60% D.M.	None	60.0	57.2	4.5		12	Fair

a The first number refers to the silo and the second to the layer. The layers are in the order in which they were removed from the silo.

b A mixture of 10 lbs. salt, 36.4 gm. Silogerm, 9.05 gm. lactose, and water to make 120 lbs. was applied at the rate of 30 lbs. per ton as recommended by the manufacturer of Silogerm.

e Palatability was judged by odor, appearance and consumption.

use of other adjuvants.   As judged by pH, odor, carotene content and palatability, alfalfa ensiled with corn and cob meal, and sweet clover in combination with green corn in the ratios of 1:1 and 3:1 gave good silages.   A fair silage was obtained from wilted alfalfa.   Poor silages were obtained from alfalfa ensiled with Silogerm and salt and with Vacatone (residual solids from the industrial fermentation of molasses).   The untreated alfalfa was very poor.   More extensive chemical analyses were performed on the alfalfa preserved with Silogerm and salt.   The ammonia nitrogen in this silage amounted to 0.70 per cent, the acetic acid to 3.32 per cent, and the butyric acid to 4.98 per cent, all calculated on the basis of dry-matter.   The volatile acids and the ammonia content of this silage were similar to the values for poor quality silage reported in previous experiments (5).

In 1942 other materials were used as preservatives (layers III–1 to III–6).   The object in these experiments was to raise the dry-matter content of the ensiled material to 30 or 35 per cent.   The fresh forage was in the late stages of maturity when ensiled.   The alfalfa layers preserved with ground or whole corn and by wilting produced fair to good silages.   The wilted forage had been rained on twice and had been wilted for 3 days, resulting in a very low carotene content.   The alfalfa layers treated with oat straw and ground wood shavings were not palatable and had characteristic straw-like and woody odors.   Dry sorghum fodder did not preserve the alfalfa, for the silage was moldy and dry, and the carotene content was low, even though the acidity was equal to that found in good silages.

Composition of silages used in longer feeding trials.   Of the many preservatives used in the palatability tests, several were tested further on a larger scale in order to include more complete feeding trials.   In 1940, three different silos were filled with the following forages: oat-and-pea mixture sown in a ratio of 4 to 1, Sudan and soybean combination in a seeding ratio of 2 to 1, and alfalfa.   Table 2 gives the kind and the amount of preservative used, as well as the analytical data on the fresh forage and the corresponding silage.   All three silages were very palatable.   However, the carotene contents of lots 3a, 3b, and 3c were low regardless of the kind and amount of preservative used.   There was no apparent explanation for the high carotene loss.

In the following year, 1941, the soybean-sorghum silage, in ratios of 1:1, 2:1, and 4:1, was of good quality, although the loss of carotene during ensiling was high in two of the lots.   Lots 5a, 5b, and 5c, which were preserved with whey powder, were rated as good silages in spite of the poor carotene preservation.   Lots 6a, 6b, and 6c, which consisted of one layer containing no preservative and two others containing different amounts of corn and cob meal, were very good silages as judged by odor and color.   However, the carotene preservation was not the same for all layers.   The layer which contained 250 pounds of corn and cob meal had very little caro-

TABLE 2

Composition of silages used in feeding trials

Lot	Year	Material	Treatment	Preservative (lbs./ton)	Dry matter Fresh forage (%)	Dry matter Silage (%)	pH	Carotene (dry basis) Fresh forage (µg./gm.)	Carotene (dry basis) Silage (µg./gm.)	Carotene loss (%)
1a	1940	Oats-peas	None		27.8	28.7	4.1	131	99	24
1b	1940	"	Phosphoric acid	15	29.1	27.4	4.0	144	130	10
1c	1940	"	"	8	27.4	25.8	3.9	149	138	7
1d	1940	"	Molasses	40	25.3	25.3	3.9	165	130	21
2a	1940	Sudan (2)–soybeans (1)	None		20.7	19.9	3.9	204	106	48
2b	1940	"	Phosphoric acid	12	20.4	17.4	4.5	266	128	52
2c	1940	"	Molasses	40	20.8	19.6	4.1	220	196	11
3a	1940	Alfalfa	Molasses	60	29.7	27.5	4.4	126	76	40
3b	1940	"	Corn and cob meal	150	30.2	33.8	4.3	160	32	80
3c	1940	"	"	200	28.8	33.5	4.3	188	90	52
4a	1941	Soybeans (1)–sorghum (1)	None		29.7	31.6	3.7	143	84	41
4b	1941	Soybeans (2)–sorghum (1)	None		32.2	30.7	4.0	143	74	48
4c	1941	Soybeans (4)–sorghum (1)	None		34.9	30.1	4.3	98	85	13
5a	1941	Alfalfa	Whey powder	10	27.4	30.6	4.5	135	56	59
5b	1941	"	"	20	28.6	28.0	4.4	135	32	76
5c	1941	"	"	30	34.3	32.7	4.3	135	68	50
6a	1941	"	None		34.6	36.2	4.5	113	7	94
6b	1941	"	Corn and cob meal	200	29.5	29.6	4.3	113	17	85
6c	1941	"	"	250	29.5	28.0	4.3	113	89	21
7	1942	"	Wilted, 40% D.M.		41.7	39.9	4.9	116	64	45
8	1942	"	Corn and cob meal	200	29.5	30.8	4.5	125	70	44
9	1943	Corn	None		22.4	21.7	3.8	107	72	33
10	1943	Alfalfa	Corn and cob meal	200	24.0	23.6	4.4	202	241	0

tene loss; 200 pounds of corn and cob meal gave very low carotene preservation; and the layer which was untreated had lost most of the original carotene. The difference in carotene preservation obtained with a variation of 50 pounds in corn and cob meal was unexpected. The alfalfa used in these experiments was ensiled in the late blossoming stage in order to raise the dry-matter content. In consequence of this more mature condition, it was low in carotene and in most lots the loss was heavy. Corn and cob meal is slow in promoting the development of lactic acid–producing bacteria, which are important in bringing about anaerobic conditions and consequent retardation of carotene oxidation. Also, a high dry-matter content is less favorable for good packing and exclusion of air from the ensiled material. With several factors involved, it is possible that in a given case irregular results may be obtained. Even with molasses, which is a much better preservative than corn and cob meal, poor preservation is encountered occasionally. In our judgment, good preservation of carotene should not entail a loss of more than 25 per cent of the carotene of the forage.

In 1942, two silos were filled with alfalfa preserved by wilting to 40 per cent dry matter (lot 7) and by adding 200 pounds of corn and cob meal per ton (lot 8). Chemical analyses of these two silages (table 2) show that good silages were obtained. The carotene loss was approximately 50 per cent. The alfalfa silage containing corn and cob meal was more readily consumed by the animals than the untreated alfalfa.

In 1943, the experiments were set up to determine the comparative value of corn and alfalfa silages for maintaining the carotene and the vitamin A content of milk. To obtain silages high in carotene content, material in the early stages of maturity was ensiled. In one silo, succulent alfalfa was preserved with 200 pounds corn and cob meal and, in another, corn in the early-dough stage was ensiled. Excellent silages (lots 9 and 10) were obtained. The data show that in order to obtain good silage, the material ensiled must be of good quality.

Milk production and change in body weight. Table 3 summarizes the data on feed consumption, milk production, and changes in body weight of animals fed the silages listed in table 2. In the 1940, 1941, and 1943 feeding experiments, each lot consisted of five cows, while in the 1942 feeding trials the lots were made up of nine cows each. The alfalfa hay used for these experiments contained from 15 to 25 micrograms of carotene per gram of dry matter. The rations for all lots, except 10 and 11, contained equivalent amounts of protein as the result of adding linseed meal to the grain mixtures of lots 1, 2, 4, and 9. From the data in table 3 it appears that the silages were of approximately equal nutritive values, as judged by milk production and body weight.

In 1942 feeding trials showed that apparently all of the corn and cob meal used as preservative for the alfalfa was available to the animal. The

TABLE 3

Feed consumption, milk production and change in body weight per cow per day

Lot	Silage fed	Silage	Alfalfa hay	Grain mixture*	Av. daily 4% F.C. milk	Weight change	Av. decline 4% F.C. milk
		lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
1940	*17 weeks feeding period*						
1	Oats-peas	42.4	9.2	11.3	27.97	+0.31	0.101
2	Sudan-soybeans	43.6	9.7	11.2	28.56	−0.06	0.088
3	Alfalfa–corn and cob meal	42.8	9.5	10.9	28.03	+0.56	0.102
1941	*16 weeks*						
4	Sorghum-soybeans	48.8	6.4	8.3	26.85	+0.029	0.105
5	Alfalfa–whey powder	47.4	6.2	8.3	25.02	−0.33	0.139
6	Alfalfa–corn and cob meal	49.1	6.4	8.3	26.88	−0.06	0.139
1942	*7 weeks*						
7	Alfalfa–wilted	31.4	5.6	10.4	30.09	−0.42	0.066
8	Alfalfa–corn and cob meal	50.1	5.5	6.1	30.16	−0.07	0.038
1943	*13 weeks*						
9	Corn	40.3	14.0	8.5	25.67	+0.80	0.065
10	Alfalfa–corn and cob meal	40.8	14.0	8.7	24.41	+0.91	0.080
11	Corn, ½; alfalfa–corn and cob meal, ½	40.4	14.0	8.7	25.02	+0.86	0.088

* Ingredients

	Lots 1, 2 and 4	Lots 3, 5 and 6	Lot 7	Lot 8	Lots 9, 10 and 11
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Ground corn	64.4	69.4	48.5	19.0	38
Ground oats	24.8	27.7	48.5	76.2	30
Bran	....	....	....	....	20
Linseed meal	9.9	....	....	....	10
Bone meal	....	1.9	2.0	2.9	1.0
Iodized salt	1.0	1.0	1.0	1.9	1.0
Calcium flour	....	....	....	....	1.0
Irradiated yeast	....	....	....	....	0.025

grain mixture fed lot 8 was adjusted to allow for the corn and cob meal used in the preservation of the alfalfa. There were no differences between the two lots, one receiving wilted alfalfa silage (lot 7) and the other silage preserved with corn and cob meal (lot 8).

Lots 9, 10, and 11, which were based on an ordinary farm ration, showed that good corn silage is equivalent to good alfalfa silage for milk production.

Carotene and vitamin A content of milks. The data for the carotene and vitamin A intake of each lot of cows on the feeding trials as well as the vitamin A potency of the milks produced are given in table 4. The milks from the cows in lots 1, 2, and 3 had a high carotene and vitamin A content as compared to the milks produced in the 1941, 1942, and 1943 trials. Milk from lots 4, 5, and 6 had only about 50 per cent of the vitamin A potency (carotene and vitamin A together) of the 1940 milks. The silages fed were low in carotene, and the carotene and vitamin A contents of the corresponding milks were below the average for winter milk. The feeding trials in 1942 showed no differences in the vitamin A potency of the milks produced by animals fed wilted alfalfa silage and those fed alfalfa preserved with corn and cob meal. Waugh *et al* (6), in comparing two groups of cows, one fed corn silage and the other alfalfa–brome grass silage, found no differences in milk production or change in body weight. However, the cows fed corn silage containing 50 micrograms of carotene per gram of dry matter produced milk exceedingly low in vitamin A potency (approximately 9 I.U. per gram of butterfat). The animals fed alfalfa–brome grass silage produced milk of vitamin A potency above the average for winter feeding.

The 1943 experiments indicated that corn silage of high carotene content was almost equivalent to excellent alfalfa silage.[3] Although the cows in lot 10 ingested approximately three times as much carotene as those in lot 9, the vitamin A potency of the milk was only 20 per cent higher than from lot 9 and no higher than that from cows ingesting about two-thirds as much carotene (lot 11). The carotene and vitamin A content of milk from lot 10 accounted for less than one per cent of the carotene intake. In lot 9, 2 per cent of the ingested carotene was accounted for in the milk. As may be seen, the amount of carotene ingested did not correlate with the vitamin A potency of the milk produced. Other undetermined factors appear to have operated. The vitamin A level of the milk for each lot remained constant for the 13 weeks of the 1943 feeding trial.

Survey of experimental farm milks. These data were obtained as part of a grass silage harvester program under farm conditions which was sponsored

[3] Another short experiment, in which cows fed a low-carotene corn silage (26 µg. per gram dry matter) were switched to a high-carotene corn silage, showed that the vitamin A potency of the milk followed the increase in carotene intake very closely. At the beginning of the experiment the vitamin A potency of the milk was 14 I.U. per gram of butterfat and, after feeding high carotene silage for 3 weeks, it rose to 30 I.U. per gram of butterfat.

TABLE 4

Carotene and vitamin A content of milks produced in feeding trials

Lot	Year	Silage	Butterfat	Carotene intake*	Carotene	Vitamin A	Vitamin A potency, I.U.	
			%	mg./day	µg./gm. butterfat	µg./gm. butterfat	per gm. butterfat	per 100 ml. milk
1	1940	Oats-peas	3.5	722	9.3–10.2 / 9.8†	8.3–8.6 / 8.5†	50	183
2	1940	Sudan-soybean	3.5	641	8.5–8.9 / 8.7	7.1–8.3 / 7.7	45	166
3	1940	Alfalfa–corn and cob meal	3.9	491	6.0–7.5 / 7.3	8.2–8.6 / 8.4	46	185
4	1941	Soybean-sorghum	3.9	611	2.1–3.4 / 2.9	2.9–5.2 / 3.9	20	84
5	1941	Alfalfa–whey powder	3.5	396	3.3–3.7 / 3.5	4.2–4.6 / 4.4	23	83
6	1941	Alfalfa–corn and cob meal	3.8	323	1.5–3.0 / 2.5	2.3–4.9 / 3.6	19	74
7	1942	Alfalfa–wilted, 40% D.M.	3.55	415	2.9–3.2 / 3.0	6.1–6.6 / 6.4	31	113
8	1942	Alfalfa–corn and cob meal	3.85	540	5.2–5.6 / 5.4	5.9–6.0 / 6.0	33	131
9	1943	Corn	4.0	414	4.1–5.8 / 4.6	5.2–7.4 / 5.6	30	124
10	1943	Alfalfa–corn and cob meal	3.6	1164	4.2–7.0 / 5.7	6.0–7.9 / 6.9	37	137
11	1943	Corn (1 part); alfalfa and cob meal (1 part)	4.2	784	3.8–5.9 / 5.4	5.2–6.5 / 5.9	33	143

* Includes carotene supplied by hay; 20 µg. per gram of dry matter was used as an average value.
† The third figure is the average of the preceding two figures.

TABLE 5

Data on experimental farm silages and milks

| Farm no. | Silage* | Silage analysis | | | Milk analysis | | | Vitamin A potency, I.U. | |
| | | pH | Dry matter | Carotene | Butterfat | Carotene | Vitamin A | | |
			%	μg./gm. dry matter	%	μg./gm. butterfat	μg./gm. butterfat	per gm. butterfat	per 100 ml. milk
1	Alfalfa	4.2	29.5	250	3.5	4.7	10.6	50	180
2	Sweet clover	3.9	29.5	75	3.7	3.4	7.0	34	130
3	Alfalfa	4.1	26.3	154	3.9	3.8	5.9	30	120
4	Alfalfa	4.2	29.3	156	4.8	4.8	5.6	30	148
5	Alfalfa	4.4	30.5	30	3.2	2.1	5.7	26	86
6	Timothy	4.1	29.3	74	3.6	1.6	5.3	24	89
7a	Corn	.....	.....	.....	5.0	8.8	3.0	27	139
7b	Alfalfa	.....	25.6	118	4.7	10.0	3.4	30	145

* All grass silages were preserved with 200 lbs. corn and cob meal with the exception of that from farm no. 4 which was preserved with molasses.

by the College of Agriculture. The analyses (table 5) show that four of the alfalfa silages were unusually high in carotene. These were ensiled during the early stages of maturity. Farm no. 1 produced milk which had a vitamin A potency equal to that of summer milk. This herd had been fed high carotene silage for 3 months previous to the time the sample was taken. It will be noted that the feeding of low-carotene silage (*e.g.*, farm no. 5) resulted in milk low in vitamin A potency.

An experiment was conducted at the La Crosse, Wisconsin, Soil Conservation Experiment Station (farm no. 7) to note the effect of replacing corn silage in an ordinary farm ration with alfalfa silage. During the last month (February) on corn silage, the vitamin A potency decreased from 32 to 27 I.U. per gram of butterfat. The cows were then switched to alfalfa silage which contained 118 micrograms of carotene per gram of dry matter. The decline stopped, and after 3 weeks the vitamin A potency reached 30 I.U. per gram of butterfat and remained at this level for a month, when the experiment was terminated.

SUMMARY

In a study of various methods of ensiling grasses and legumes, preservation by the addition of 200 pounds corn and cob meal per ton gave a very palatable silage, although carotene preservation was not as good as with some other methods, *e.g.*, molasses.

No significant differences were found in milk production and change in body weight when the lots for any one feeding period were compared.

A comparison of good corn and alfalfa silages indicated that both silages had apparently equivalent feed value on the basis of milk production and change in body weight. The vitamin A potency of the milk produced from these two silages varied by only 20 per cent. Alfalfa silage high in carotene generally increased and maintained the carotene and vitamin A level in winter milk.

REFERENCES

(1) BERL, SOLL, AND PETERSON, W. H. Determination and Content of Carotene and Vitamin A in Wisconsin Butter. Jour. Nutr., 26: 527–538. 1943.

(2) HEGSTED, D. M., PORTER, J. W., AND PETERSON, W. H. Determination of Carotene in Silage. Indus. and Engin. Chem., Analyt. Ed., 11: 256–258. 1939.

(3) JOHNSON, B. C., PETERSON, W. H., HEGSTED, D. M., AND BOHSTEDT, G. A Study of Various Methods of Preserving Legumes and other Forages by Ensiling. Jour. Agr. Res., 62: 337–348. 1941.

(4) OLSON, F. R., HEGSTED, D. M., AND PETERSON, W. H. Determination of Carotene and Vitamin A in Milk. Jour. Dairy Sci., 22: 63–66. 1939.

(5) PETERSON, W. H., BIRD, H. R., AND BEESON, W. M. Chemical Changes in Making A.I.V. Alfalfa Silage and Nutritive Qualities of Milk Produced Therefrom. Jour. Dairy Sci., 20: 611–623. 1937.

(6) WAUGH, R. K., HAUGE, S. M., WILBUR, J. W., AND HILTON, J. H. A Comparison of Alfalfa-Brome Grass Silage and Corn Silage for Dairy Cows. Jour. Dairy Sci., 26: 921–928. 1943.

(7) Wisconsin Agricultural Experiment Station. Ensiling Partially Dried Alfalfa. Wis. Agr. Expt. Sta. Ann. Rpt. 40(1922–23): 362–376. 1924.

THE EFFECT OF SUPPLEMENTARY VITAMINS ON BLOOD COMPOSITION, LIVER STORAGE, AND INCIDENCE OF SCOURS IN CALVES

J. W. HIBBS AND W. E. KRAUSS

Ohio Agricultural Experiment Station, Wooster, Ohio

Following the reports by Wisconsin workers (4, 8) that supplementary vitamin feeding is beneficial in preventing calf scours, much interest has developed regarding the possibilities in vitamin supplementation for calves. Most previous work has been done with calves maintained on skim milk or calves from dams known to be on a low level of vitamin A intake. We, therefore, undertook to determine the effect of two systems of vitamin supplementation on the blood picture, liver storage, and scour incidence of calves maintained under normal herd conditions.

In these experiments each calf was allowed to nurse before the dam was milked out. The calves remained on their dams for at least 3 days and were then pail-fed on whole milk. Hay and grain were fed beginning at 2 weeks of age. Calves of both the Jersey and Holstein breeds were included in this experiment and the blood and liver data of both breeds are combined in the results.

Blood plasma vitamin A and carotene were determined by the method of Kimble (2). Liver vitamin A and carotene were determined by using the extraction procedure of Guilbert and Hart (1). Blood plasma ascorbic acid was determined by the macromethod of Mindlin and Butler (6). All colorimetric readings were made using an Evelyn photoelectric colorimeter.

EXPERIMENT 1

The first experiment was carried out during the late winter and early spring months of 1945, before the pasture season. Alternate calves were placed in Group I (control) and Group II (experimental). Group I (fifteen calves) received a placebo capsule[1] containing a biologically inactive oil. Group II (fifteen calves) received one multivitamin capsule daily for the first 20 days. These capsules contained 10,000 USP units of vitamin A, 300 USP units of vitamin D, 50 mg. of niacin, and 250 mg. of ascorbic acid.

Blood samples were drawn for analysis of plasma vitamin A, carotene, and ascorbic acid on the same day each week from all calves under 31 days of age. In averaging the results, all determinations made on the first, second, and third days were averaged separately. All determinations made between the fourth and eleventh days were grouped together and considered as the seventh

115

day. Similar groupings of the data were made centering on the fourteenth, twenty-first, and twenty-eighth days. Total vitamin A liver storage was determined on four male calves in Group I and on five male calves in Group II at 21 days of age.

The average results of the blood and liver analyses are presented in figure 1. The vitamin A liver storage indicated is due to vitamin A alone. A small carotene storage was found which amounted to an average of 813 USP units per liver in the nine calves. Plasma vitamin A reached a peak on the
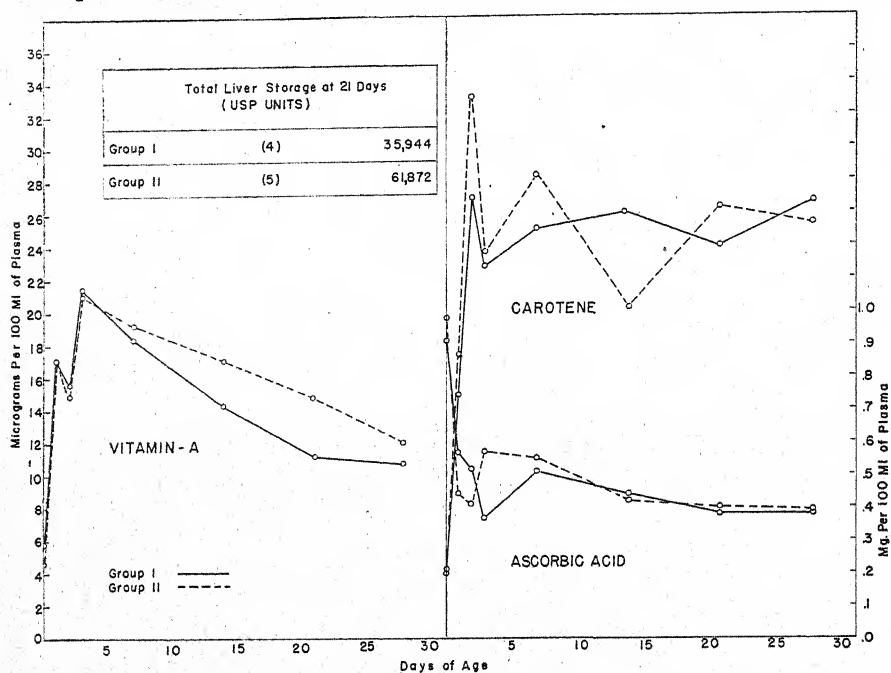
FIG. 1. The effect of daily supplementary vitamin feeding on the blood plasma vitamin A, carotene, and ascorbic acid, and on vitamin A liver storage in calves.

third day and then declined rapidly. No difference in the groups was observed until after the third day, when it was shown that Group II (experimental) did not decline in blood vitamin A as rapidly as did Group I (control). There was a decline in blood vitamin A regardless of supplementary feeding. Liver storage at 21 days in Group II was found to be nearly double that of Group I, indicating marked liver storage due to the supplemental vitamin A. Greater individual variations were found in the plasma carotene levels; however, no marked difference between the two groups is indicated.

Plasma ascorbic acid was found to be extremely high immediately after birth. The initial high level rapidly dropped so that normal levels were usually found within 24 hours. No beneficial effect of feeding ascorbic acid

was observed except that Group II showed a slight increase over Group I between the third and seventh days.

Although a considerable number of calves had scours, no difference was noted in the incidence between Group I and Group II.

EXPERIMENT 2

To determine the effects of feeding massive doses of vitamin A at less-frequent intervals, the following experiment was carried out from February through April, 1946. Holstein and Jersey calves born in the Experiment Station herds were assigned to one of three groups. Group I (ten calves)
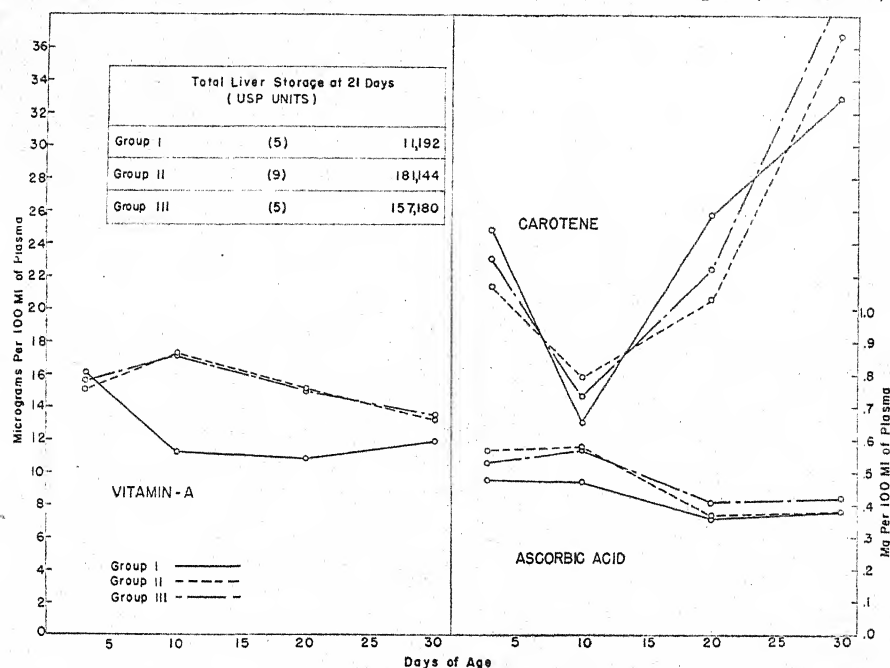
FIG. 2. The effect of feeding 250,000 USP units of vitamin A on the third and tenth days, plus 50 milligrams of niacin daily for 20 days, on the blood plasma vitamin A, carotene, and ascorbic acid, and on liver storage in calves.

served as a control group. Group II (thirteen calves) received a capsule containing 250,000 USP units of vitamin A on the third and tenth days after birth. Group III (twelve calves) received the same vitamin A supplement and, in addition, were given 50 mg. of niacin in a gelatin capsule daily for the first 20 days. Blood samples were drawn for analysis for plasma vitamin A, carotene, and ascorbic acid on the third, tenth, and twentieth days for all calves and also on the thirtieth day for the females. The male calves in each group were sacrificed on the twenty-first day and the total vitamin A liver storage determined. The average results of the blood and liver storage data are shown in figure 2.

It is evident that the supplemental vitamin A feeding resulted in the maintenance of a higher blood level in Groups II and III. However, the most marked effect of vitamin A feeding showed up in the amounts stored in the liver. Nearly ten times as much vitamin A was stored in the livers of the experimental group as in the control group. No difference was noted between Groups II and III in the blood level of vitamin A or in liver storage. Thus, the addition of niacin did not raise the blood level of vitamin A or increase liver storage at this level of vitamin A intake. The average liver carotene value of the nineteen male calves in the three groups was 633 USP units per liver.

Although the blood carotene followed a somewhat different pattern in experiment 2 than in experiment 1, no difference among the three groups is detectable. No differences were noted among the groups regarding the average ascorbic acid level. The incidence of scours was the same in all three groups.

DISCUSSION

In these experiments, no lowering of scours incidence was noted which could be attributed to supplemental vitamin feeding. This is in general agreement with the work of Norton *et al.* (7).

The incidence of scours was much higher in the Jersey calves than in the Holsteins. The average plasma vitamin A of the Jersey calves was lower than that of the Holsteins. Possibly this is due to the higher incidence of scours in the Jersey calves. The average carotene level of Holstein plasma was lower than that of the Jerseys. This observation is in agreement with the results reported by Moore (5). Plasma vitamin A and carotene, as well as vitamin A liver storage, were reduced in calves that had severe scours.

These data show that the decrease in plasma vitamin A of calves during the first few weeks can be offset to a considerable extent by feeding supplemental vitamin A according to either of these two systems. It is of interest that Sutton and Kaeser (9) have shown that, when colostrum feeding was extended for 7 days, the blood vitamin A level at 21 days was nearly identical with that of calves that received 10,000 units of vitamin A daily for 21 days.

No linear correlation between plasma vitamin A and liver storage was observed when liver storage was high. At low liver storage levels the plasma vitamin A values were a good index of liver storage. In general, data reported by Lewis and Wilson (3) confirm these observations. However, the experimental procedures are not sufficiently comparable for direct comparison of the data.

It is questionable how much benefit to the health of the calf is derived from excessively high liver storage or increases in blood vitamin A over the normal levels, when a normal ration is fed. In large-scale field trials conducted in Ohio and Michigan, the results of which are to be published else-

where, no lowering of scour incidence could be attributed to the feeding of supplemental vitamin A. It is reasonable to believe, however, that calves with a high vitamin A storage would be able to withstand periods of low vitamin A intake or impaired absorption much better than calves raised without supplementary vitamin A. Since both the plasma vitamin A content and liver storage of calves that had severe scours were extremely low, the administration of supplementary vitamin A to such calves would seem to be indicated in order to counteract the subnormal levels. Calves with severe scours often had plasma vitamin A levels as low as 4.5 micrograms per 100 ml. In Group III one calf, which had severe scours for 8 days, had a vitamin A liver storage value of 54,420 USP units at 21 days, whereas the average value for the group was 157,180 USP units. This calf had a vitamin A blood plasma level of 4.4 micrograms per 100 ml. at 20 days of age. The average blood vitamin A level for the group at this age was 13.9 micrograms per 100 ml.

SUMMARY AND CONCLUSIONS

1. Calves fed extra vitamin A, either 10,000 USP units daily for 20 days or 250,000 USP units on the third and tenth days, maintained a higher blood plasma vitamin A level after the third day than did their controls.

2. Liver vitamin A storage was increased with increased vitamin A intake. Plasma vitamin A and liver storage were not closely correlated except at low levels of liver storage.

3. The daily addition of 50 mg. of niacin when 250,000 USP units of vitamin A were fed on the third and tenth days had no effect on blood plasma or liver storage vitamin A values.

4. Except for a slight increase between the third and seventh days, no effect was observed on the plasma ascorbic acid content when 250 mg. of ascorbic acid were fed daily for 20 days.

5. No significant effect on lowering the incidence or severity of scours could be detected when supplementary vitamins were added to the normal ration according to the procedures described.

It is concluded that routine supplementary feeding of vitamin A, ascorbic acid, and niacin to calves during the first few weeks following birth is of doubtful value in preventing scours. The feeding of supplementary vitamin A at the rate of 10,000 USP units daily for 20 days or 250,000 USP units on the third and tenth days will help overcome any deficiency of vitamin A intake resulting from inadequate feeding of colostrum and whole milk, from impaired absorption, or from subsequent feeding of poor-quality hay.

REFERENCES

(1) GUILBERT, H. R., AND HART, G. H. Storage of Vitamin A in Cattle. Jour. Nutr., 8: 25–44. 1934.
(2) KIMBLE, M. S. The Photoelectric Determination of the Vitamin A and Carotene in Human Plasma. Jour. Lab. and Clin. Med., 24: 1055–1065. 1939.

120

(3) LEWIS, J. M., AND WILSON, L. T. Vitamin A Requirements in Calves. Jour. Nutr., 30: 467–475. 1945.

(4) LUNDQUIST, N. S., AND PHILLIPS, P. H. Certain Dietary Factors Essential for the Growing Calf. Jour. Dairy Sci., 26: 1023–1030. 1943.

(5) MOORE, L. A., AND BERRY, M. H. The Effect of Colostrum on the Vitamin A and Carotene Content of Blood Plasma of New-Born Calves. Jour. Dairy Sci., 27: 867–873. 1944.

(6) MINDLIN, R. L., AND BUTLER, A. M. The Determination of Ascorbic Acid in Plasma: A Macromethod and a Micromethod. Jour. Biol. Chem., 122: 673–686. 1938.

(7) NORTON, C. L., EATON, H. D., LOOSLI, J. K., AND SPIELMAN, A. A. Controlled Experiments on the Value of Supplementary Vitamins for Young Dairy Calves. Jour. Dairy Sci., 29: 231–238. 1946.

(8) PHILLIPS, P. H., LUNDQUIST, N. S., AND BOYER, P. D. The Effect of Vitamin A and Certain Members of the B-complex upon Calf Scours. Jour. Dairy Sci., 24: 977–982. 1941.

(9) SUTTON, T. S., AND KAESER, H. E. Some Physiological Effects of Extending the Colostrum Feeding Period of Dairy Calves. Jour. Dairy Sci., 29: 13–26. 1946.

COPPER AND IRON IN THE BLOOD SERUM OF DAIRY COWS[1, 2, 3]

GENNARD MATRONE

U. S. Plant, Soil, and Nutrition Laboratory, Ithaca, New York

WALTER J. PETERSON, HARTLEE M. BAXLEY, AND CLAUDE D. GRINNELLS

Department of Animal Industry, North Carolina State College, Raleigh

The usefulness of blood or blood serum levels of copper, iron, hemoglobin, and cell volume as indications of nutritional deficiencies of cattle grazing in areas deficient in certain micronutrient elements has been shown by the work of various investigators (1, 2, 3, 5, 13). The utility of this blood picture tool, however, is dependent on a knowledge of the normal levels and variabilities of these blood characteristics. A review of the literature reveals no general agreement in the results reported for blood serum copper and iron levels for dairy cows. In view of this, the work reported here was undertaken as preliminary to a project designed to study certain deficiencies of micronutrient elements in some coastal areas of North Carolina. Hemoglobin and cell volume were run concurrently with iron and copper and also are reported.

Most of the published copper values on bovine blood are reported on whole blood rather than on blood serum. Tompsett (16), however, presented data showing that the copper of the blood was distributed evenly between the plasma and the corpuscles of the blood for man, sheep, ox, pig, and horse. The data of Kehoe *et al* (9) also show the copper content of the corpuscles and plasma of blood to be of the same order. In this laboratory, no significant difference was found in copper content between blood serum badly contaminated with red cells and serum with little or no red-cell contamination. The copper content for apparently normal bovine blood or blood serum, as determined by various workers, is shown in table 1. Blood serum iron values for dairy cows are meager.

MATERIALS AND METHODS

The principal difficulty in determining iron in blood serum is to obtain a preparation completely free from hemoglobin and other forms of organic iron. This problem has been discussed by Kitzes *et al* (10), and a method is presented which appears to give satisfactory results. In our work, the method of Kitzes was used to prepare the blood serum filtrate for the iron

determination. Instead of a,a'-bipyridine, however, o-phenanthroline was used for the color reagent, as outlined by Parks, et al (14).

In some preliminary work, it was found that wet-ashed serum gave higher copper values than blood serum filtrate prepared according to Kitzes. Typical values obtained were: wet-ashed serum, 103 μg. of copper per 100 ml. of serum; and blood serum filtrate, 68 μg. of copper per 100 ml. of serum. These values are an average of twelve samples in duplicate. In view of these results, a wet-ash method was adopted. The blood serum was wet-ashed with concentrated nitric and perchloric acid. Essentially the method outlined by Parks et al (14) was used for the determination of copper. The method of Shenk et al (15) was used for the hemoglobin determination, and the Sanford Magath hematocrit tube for cell volume.

The experiment was designed to study the influence of breed, age, and time on the levels of the blood constituents under study. Blood was obtained

TABLE 1

Normal bovine blood or blood serum values obtained by various workers

Author	Animal	Type of blood	Copper
			μg./100 ml.
McHargue (12)	Ox	Whole blood	140
Guillemet (6)	Cow	Serum	58–82
Tompsett (16)	Ox	Whole blood and serum	192–223
Bennetts et al (3)	Cow	Whole blood	30–100
Beck (2)	Cow	Whole blood	70–170
Cunningham (5)	Cow	Whole blood	Mean of 100

from Ayrshire, Holstein, Guernsey, and Jersey cows in the college herds. The animals were subdivided into four age classifications: 4 to 6 months, 12 to 18 months, 2 to 2.5 years, and 4 to 7 years, with one animal from each breed in each age class. Blood from each animal was analyzed at four different times: January 2, February 14, March 6 and March 20. The four different dates will be referred to as Periods I, II, III, and IV, respectively. The cows were barn-fed for all periods except Period IV, when they were on pasture. Throughout the experimental period, all the animals received hay and a 17 per cent protein concentrate mixture. In addition, the animals in the age groups 2 to 2.5 years and 4 to 7 years received corn silage. The calves, 4 to 6 months of age, received a supplement of milk during most of the first two periods and a supplement of calf manna during the remainder of the experiment.

EXPERIMENTAL DATA

The means for iron, copper, hemoglobin, and cell volume, classified according to the ages of the animals, together with their standard errors, are presented in table 2.

TABLE 2

Effect of age on the blood level of indicated characteristic

Blood characteristics	Age				Standard error of a mean $S_{\bar{x}}$	Significance of age effect*
	4–6 months	12–18 months	2–2½ years	4–7 years		
Serum iron, µg./100 ml.	171.2	155.0	157.3	162.4	± 12.0	o
Serum copper, µg./100 ml.	94.5	85.0	109.9	113.8	± 6.2	hs
Hemoglobin, gm./100 ml.	9.08	9.72	10.06	10.64	± 0.26	hs
Cell volume, % of total	29.37	30.92	32.40	34.53	± 1.27	o

* o = not significant ($P > 0.05$).
  s = significant ($P \leqq 0.05$).
  hs = highly significant ($P \leqq 0.01$).

As indicated in the table, no evidence was obtained that serum iron changes with age. Copper and hemoglobin, however, changed significantly with age, the higher values being observed with advancing age. Although cell volume increased with advancing age, this effect was not statistically significant. On the other hand, there was a positive and significant correlation ($r = + 0.9986$) between hemoglobin and cell volume from age group to age group. It might be concluded, therefore, that perhaps both hemoglobin and cell volume increase with advancing age.

The influence of breed on the blood levels of the characteristics studied is summarized in table 3.

No evidence was obtained that the blood characteristics differ among breeds. In this connection Tompsett (16), in his copper studies of various species, reports "the copper contents of sheep, ox, pig, horse, and guinea pig are of the same order as that of human blood." McCay (11) and Brooks *et al* (4), working with the four breeds used in this study, also found no difference in hemoglobin levels among breeds.

The effect of period on the blood levels of the characteristics is presented in table 4.

TABLE 3

Effect of breed on the blood level of the indicated characteristic

Blood characteristics	Breed				Standard error of a mean ($S_{\bar{x}}$)	Significance of breed effect*
	Ayrshire	Holstein	Guernsey	Jersey		
Serum iron, µg./100 ml.	130.8	168.3	163.7	183.1	± 12.0	o
Serum copper, µg./100 ml.	93.6	92.5	99.1	115.6	± 6.2	o
Hemoglobin, gm./100 ml.	10.39	9.50	9.94	9.67	± 0.26	o
Cell volume, % of total	32.14	29.78	34.45	30.85	± 1.27	o

* o = not significant ($P > 0.05$).

TABLE 4

Effect of period on the blood level of the indicated characteristics

| Blood characteristics | Period | | | | Standard error of a mean ($S_{\bar{w}}$) | Significance of period effect[*] |
	I 1/2/46	II 2/14/46	III 3/6/46	IV 3/20/46		
Serum iron, μg./100 ml. ...	130.3	169.2	162.6	183.9	± 6.8	hs
Serum copper, μg./100 ml.	87.8	101.2	116.3	95.2	± 4.6	hs
Hemoglobin, gm./100 ml.	10.07	9.93	10.06	9.44	± 0.17	s
Cell volume, % of total	....	30.94	31.54	32.94	± 0.75	o

[*] o = not significant ($P > 0.05$).
   s = significant ($P \leqq 0.05$).
   hs = highly significant ($P \leqq 0.01$).

As illustrated by table 4, period had a pronounced effect on all measures except cell volume. Possibly, if cell volume had been measured in Period I, it also would have shown a significant period variation. This experiment was not designed to sort out and find the causes of this period variation. Presumably, however, such factors as nutritional status of the animal, season of the year, and post-absorptive state of the animal are contributing to period variations. For example, Hemmeler (8) reports that in humans the serum iron is highest in the morning, averaging 127 μg. per 100 ml., and lowest in the evening, averaging 82 μg. per 100 ml. Hazleton (7) administered single doses of iron salts to rats and found the maximal level of serum iron to occur two to three hours after administration. Bennetts *et al* (3) made monthly analyses of blood copper of healthy cows from April through December and reported a low of 30 to 50 μg. in April and a high of 60 to 100 μg. per 100 ml. in September.

In table 5 are presented the over-all means and their coefficients of variation. It may be pointed out that the observations which make up the general or over-all means are subject to both the controlled variation or experimental error due to technique, age, breed, and period, as well as uncontrolled variation. The coefficients of variation presented in table 5 are measures of total variation; they include unbiased estimates of both controlled and uncontrolled variation for dairy cows under the conditions of the experiment.

The error due to technique, such as sampling, manipulation, reading, etc.,

TABLE 5

Grand means of the indicated blood characteristics and their coefficients of variation

Blood characteristic	Grand mean	Coefficient of variation
		%
Serum iron, μg./100 ml.	161.5	26.75
Serum copper, μg./100 ml.	100.1	23.93
Hemoglobin, gm./100 ml.	9.87	10.20
Cell volume, % of total	31.8	13.07

was less than 5 per cent for serum iron and copper and less than 2 per cent for hemoglobin.

No evidence was obtained that iron and copper are correlated in any way. On the other hand, hemoglobin and cell volume, in general, were correlated positively. The only deviation from this observation was the negative correlation which existed among periods (-0.8812). This negative correlation was not significant, but suggested that the factors causing the blood picture to change from period to period affect cell volume and hemoglobin in opposite manners.

SUMMARY

1. The means for serum iron, serum copper, hemoglobin, and cell volume in sixteen dairy animals were 100 µg. per 100 ml., 162 µg. per 100 ml., 9.9 gm. per 100 ml., and 32 per cent of total volume, respectively. The errors of estimate are given.

2. No evidence was obtained that serum iron changed with age. Serum copper and hemoglobin changed significantly with advancing age. The copper values ranged from about 90 µg. per 100 ml. in calves to 114 µg. in 4- to 7-year-old cows, and the hemoglobin increased from 9.08 to 10.64 gm. per 100 ml. of blood in these age classes.

3. There was no significant difference among breeds with respect to any of the measurements.

4. For serum iron, serum copper, and hemoglobin, there were pronounced variations from period to period. These variations were irregular with ranges as follows: serum iron, 130.3 to 183.9 µg. per 100 ml.; serum copper, 87.3 to 116.3 µg. per 100 ml.; and hemoglobin, 9.44 to 10.07 gm. per 100 ml.

5. No evidence was obtained that serum iron and serum copper are correlated in any way. Hemoglobin and cell volume, in general, were correlated positively.

REFERENCES

(1) BALTZER, O. C., KELLHAM, B. J., DUNCAN, C. W., AND HUFFMAN, C. F. A Cobalt Deficiency Disease Observed in Some Michigan Cattle. Mich. Agr. Expt. Sta. Quart. Bul., 24: 68–70. 1941.

(2) BECK, A. B. The Blood Copper of Sheep and of Cows. Austral. Jour. Expt. Biol. Med. Sci., 19: 249–254. 1941.

(3) BENNETTS, H. W., BECK, A. B., HARLEY, R., AND EVANS, S. T. Falling Disease of Cattle in the Southwest of Western Australia. Austral. Vet. Jour., 17: 85–93. 1941.

(4) BROOKS, H. J., AND HUGHES, J. S. The Hemoglobin Content of the Blood of Dairy Cattle. Jour. Nutr., 5: 35–38. 1932.

(5) CUNNINGHAM, I. J. Copper Deficiency in Cattle and Sheep. Occurrence and Control. New Zeal. Jour. Agr., 69: 559–569. 1944.

(6) GUILLEMET, R. Content of Copper in Blood Serum. Compt. rend. soc. biol., 108: 32–33. 1931.

(7) HAZLETON, L. W., AND GODFREY, E. A. M. Absorption of Single Doses of Iron. Jour. Pharmacol. and Expt. Ther., 83: 158–166. 1945.

(8) HEMMELER, G. Metabolism of Iron; Diurnal Fluctuations in Serum Iron. Helvetica med. acta., 11: 201–207. 1944.

(9) KEHOE, R. A., CHOLAK, J., AND STORY, R. V. A Spectrochemical Study of the Normal Ranges of Concentration of Certain Trace Metals in Biological Materials. Jour. Nutr., 19: 579–592. 1940.

(10) KITZES, G., ELVEHJEM, C. A., AND SCHUETTE, H. A. The Determination of Blood Plasma Iron. Jour. Biol. Chem., 155: 653–660. 1945.

(11) McCAY, C. M. The Hemoglobin and Total Phosphorus in the Blood of Cows and Bulls. Jour. Dairy Sci., 14: 373–378. 1931.

(12) McHARGUE, J. S. The Association of Copper with Substances Containing the Fat-Soluble A Vitamins. Amer. Jour. Physiol., 72: 583–594. 1925.

(13) NEAL, W. M., AND BECKER, R. B. The Hemoglobin Content of the Blood of Healthy and Anemic ''Salt-Sick'' Cattle. Jour. Agr. Res., 46: 557–563. 1933.

(14) PARKS, R. Q., HOOD, S. L., HURWITZ, C., AND ELLIS, G. H. Quantitative Chemical Microdetermination of Twelve Elements in Plant Tissue. Indus. and Engin. Chem., Analyt. Ed., 15: 527–533. 1943.

(15) SHENK, J. H., HALL, J. L., AND KING, H. H. Spectrophometric Characteristics of Hemoglobins. I. Beef Blood and Muscle Hemoglobins. Jour. Biol. Chem., 105: 741–752. 1934.

(16) TOMPSETT, S. L. CCV. The Copper Content of Blood. Biochem. Jour., 28: 1544–1549. 1934.

ASSOCIATION ANNOUNCEMENT
ANNUAL MEETING, GUELPH, ONTARIO, CANADA,
JUNE 24-26, 1947

Members who wish to present original papers at the annual meeting should send the titles to the member of the Program Committee who represents the section before which the paper is to be given. All titles must reach the Committee before March 25, but earlier receipt will be of real value in arranging the best program. All communications regarding general plans for the Annual Meeting should be addressed to the chairman of the General Program Committee.

Manufacturing Section: C. L. Hankinson
North American Creameries, Inc.
2035 Rand Tower
Minneapolis 2, Minnesota

Production Section: Dwight M. Seath
University Station
Baton Rouge, Louisiana

Extension Section: W. T. Crandall
Cornell University
Ithaca, New York

General Program Chairman: J. A. Nelson
Department of Dairy Industry
Montana State College
Bozeman, Montana

JOURNAL OF DAIRY SCIENCE

COMPARISON OF SUCROSE, HIGH CONVERSION CORN SIRUP, AND DEXTROSE IN THE PRESERVATION OF PEACHES BY THE FROZEN-PACK METHOD FOR USE IN ICE CREAM

P. H. TRACY,[1] JOHN J. SHEURING,[1] AND M. J. DORSEY[2]

University of Illinois, Urbana, Illinois

The use of frozen-pack fruits in the ice cream industry has been gaining in popularity in recent years. This has been due largely to the superior flavor of the frozen fruit as compared with the canned. The favorable experiences with frozen foods gained during World War II undoubtedly will place even greater emphasis upon the method of preserving and transporting fruits in frozen form.

While many ice cream manufacturers prepare much of their own pack of peaches, a considerable portion of their supply is purchased from commercial dealers. The peeled peaches are sliced or made into puree and mixed with sugar (cane or beet) in varying proportions, though three parts of fruit to one part of sugar commonly is used. This mixture then is placed in containers, rapidly frozen and stored at temperatures below zero degrees Fahrenheit until used. The sugar shortage during World War II, however, made it important that some consideration be given to the use of other types of sweetening agents, such as dextrose (corn sugar) and corn sirup, in the preparation of frozen fruit to be used in ice cream.

PROCEDURE

The studies were made during late 1941 and early 1942. The fruit was obtained from the peach-breeding plots of the University of Illinois experimental farms at Olney, Illinois, and represents the 1941 crop. The types chosen for study, as listed in table 1, are eight of the most promising selections in the Station peach-breeding project. Observations were made of the flavor, degree of ripeness, and texture of the fruit when received. The peaches were immersed in boiling water for a period of 30 seconds, followed by dipping into cold water. After the fruit skins were removed and the

peaches halved and stoned, the fruit was immersed in ice water until packing, which was accomplished within 1 hour after the hot-water immersion.

The fruit was packed in pint paper cartons, using various combinations and types of sugar. Immediately after packing, the cartons were placed in a 40° F. room for a period of 2 hours; the fruit was shaken at intervals to aid in the blending of sugar and fruit. The cartons of fruit then were stored at a temperature of approximately −2° to −20° F. for periods of 4 to 8 months. At the end of the storage period, the samples were removed to a 40° F. room and permitted to thaw slowly, after which they were judged for color, flavor, texture, and sugar crystallization.

TABLE 1

Varieties, flavor and degree of ripeness of peaches when packed

Variety (cross)	Flesh color	Quality	Acid*	Degree of ripeness	Date ripe
Heath × Marigold–K ...	White	Good	....	Slightly green	Aug. 5
Heath × Marigold	White	Good	....	Slightly green	Aug. 4
J. H. Hale × Gage–K50	Yellow	Good	1.65	Slightly green	Aug. 15
Elberta × Ea. Elberta...	Yellow	Very good	0.48	Well ripened	Aug. 22
J. H. Hale × Gage	Yellow	Very good	0.76	Well ripened	Aug. 22
Gage × Elberta	Yellow	Excellent	0.63	Very ripe	Aug. 21
J. H. Hale × Elberta ...	Yellow	Good	0.72	Well ripened	Aug. 28
Elberta × Southhaven ...	Yellow	Good	0.49	Very ripe	Sept. 1

* Data on acid content supplied by Dr. R. V. Lott. Values represent percentage malic acid in the fruit juice.

Cane sugar, enzyme converted corn sirup,[3] and dextrose hydrate were the sweetening agents used in the preparation of the experimental packs.

EXPERIMENTAL RESULTS

Heath × Marigold–K

This variety of peach is white. It was packed using fruit-sugar concentrations of (2 + 1), (2.5 + 1), and (3 + 1). The following combinations of sweetening agents were used in the three concentrations listed above:

1. 100 per cent sucrose
2. 100 per cent dextrose
3. 100 per cent enzyme converted corn sirup
4. 25 per cent sucrose plus 75 per cent dextrose
5. 50 per cent sucrose plus 50 per cent dextrose
6. 75 per cent sucrose plus 25 per cent dextrose
7. 25 per cent sucrose plus 75 per cent corn sirup
8. 50 per cent sucrose plus 50 per cent corn sirup
9. 75 per cent sucrose plus 25 per cent corn sirup
10. 25 per cent dextrose plus 75 per cent corn sirup

[3] This product will be referred to as corn sirup, and contained approximately 18% moisture, 10% dextrins, 41% maltose and higher sugars, and 31% dextrose.

11. 50 per cent dextrose plus 50 per cent corn sirup
12. 75 per cent dextrose plus 25 per cent corn sirup

The peaches were slightly green but had a fair flavor when packed.   The fruit was judged after approximately 5 months of storage.

The (2+1), (2.5+1), and (3+1) packs were placed in that order when judged for color.   Of the packs prepared with a single type of sweetening agent, the sucrose samples had the best color, the packs prepared with corn sirup were second best, and the dextrose samples had the least desirable color, being very brown.   The color of the peaches using the different combinations of sucrose and corn sirup was about the same, though some preference was shown for those packs prepared with 50 per cent corn sirup and 50 per cent sucrose in a concentration of two parts of fruit to one part of sugar.

The best-flavored packs were the (2+1), (2.5+1), and (3+1) in that order.   Of the packs prepared with a single sweetening agent, the sucrose-packed fruits were best, the corn sirup packs were second best, and the dextrose-packed samples were third.   Considering all lots, the samples having the best flavor contained 50 per cent corn sirup plus 50 per cent sucrose in a concentration of two parts of fruit to one part of sugar.

Dextrose was most soluble in the (3+1) packs.   It was less soluble in combination with corn sirup than with sucrose.   There was evidence of crystallization of the dextrose in all of the corn sirup and dextrose combinations.

Heath × Marigold

This variety of peach was packed using fruit-sugar concentrations of (2+1), (2.5+1), and (3+1).   The same combinations of sucrose, dextrose, and corn sirup were used as in the previous experiment.

The (2+1), (2.5+1), and (3+1) packs were placed in that order when judged for color.   The corn sirup pack had the best color of all lots containing a single sweetening agent, followed by the sucrose pack and the dextrose pack.   The all-dextrose pack was very brown.   The colors of the peaches in combinations of corn sirup and sucrose were about the same except the 50 per cent sucrose and 50 per cent corn sirup sample in a (2.5+1) concentration, which was the best of all packs, followed by the same sweetening agent combination in a (2+1) pack.

The best-flavored packs were the (2+1), (2.5+1), and (3+1), in that order.   Of the packs containing a single sweetening agent, the sucrose-packed fruits were best, followed by corn sirup and dextrose sugar packs in the order named.   The best-flavored fruit of the entire pack was the 75 per cent sucrose plus 25 per cent corn sirup sample in a (2.5+1) concentration, followed by the sample containing 25 per cent sucrose plus 75 per cent corn sirup in a (2+1) concentration.   There was evidence of crystallization in all packs where dextrose was used.

Gage × Elberta

This variety of peach was packed using fruit-sugar concentrations of
$(2+1)$, $(3+1)$, and $(4+1)$.

The sweetening agents used in this pack were added in the form of sirup
and were compared with samples packed using sugar and corn sirup added
on a weight basis as follows:

1. 100 per cent sucrose
2. 100 per cent corn sirup
3. 50 per cent corn sirup plus 50 per cent sucrose
4. 40 per cent corn sirup
5. 50 per cent corn sirup
6. 60 per cent corn sirup
7. 40 per cent sucrose sirup
8. 50 per cent sucrose sirup
9. 60 per cent sucrose sirup
10. 20 per cent sucrose sirup plus 20 per cent corn sirup
11. 25 per cent sucrose sirup plus 25 per cent corn sirup
12. 30 per cent sucrose sirup plus 30 per cent corn sirup

These peaches were very ripe and had an excellent flavor when packed.
The fruit was judged after 4 months of storage.

The $(2+1)$, $(3+1)$, and $(4+1)$ packs were placed in that order when
judged for color. In those samples packed with sirup, the best-colored
fruits were obtained using sirups of high sugar concentrations. Of the
samples containing a single sweetening agent, the sucrose pack had the best
color, followed by the 100 per cent corn sirup pack. Both packs were of
excellent color, however.

The best-flavored packs were those of a $(2+1)$ concentration, followed by
the $(3+1)$ and the $(4+1)$ packs. The $(4+1)$ packs all were bitter. The
all-sucrose pack had the best flavor, followed by the 50 per cent sucrose plus
50 per cent corn sirup pack, which was second, and the 100 per cent corn
sirup pack, which was third. Of the packs using sirup, the best-flavored
samples were prepared with sirups of high (60 per cent) sugar concentra-
tion.

J. H. Hale × Gage

This variety of peach was packed using fruit-sugar concentrations of
$(1+1)$, $(2+1)$, $(3+1)$, and $(4+1)$. The sweetening agents used were
added in the form of a 50 per cent sirup and were compared with both dry
sucrose and 100 per cent corn sirup as follows:

1. 100 per cent sucrose—dry
2. 100 per cent corn sirup

3. 50 per cent corn sirup plus 50 per cent sucrose (heated
    to boiling and cooled before adding to peaches)
4. 50 per cent corn sirup plus 50 per cent sucrose (mixed
    cold before adding)
5. 50 per cent corn sirup plus 50 per cent sucrose (mixed
    as added to peaches)

These peaches were well ripened when packed and had a good flavor.
The fruit was judged after 5 months of storage.

The pack in general had a good appearance, with the color becoming
slightly less desirable as the concentration of sugar sirup decreased. The
all-sucrose pack had the least desirable color. The (1 + 1) pack resulted
in a slightly pulped fruit. All of the samples lacked flavor, with no dis-
tinct differences noticeable. In general the pack was not satisfactory.

Elberta × Early Elberta

This variety of peach was packed using fruit-sugar concentrations of
(1 + 1), (2 + 1), (3 + 1), and (4 + 1). The sweetening agents used were
added in the form of sirup or on a dry basis in the following combinations:

1. 100 per cent sucrose—dry
2. 100 per cent corn sirup
3. 50 per cent corn sirup plus 50 per cent sucrose (heated
    to boiling and cooled before adding)
4. 50 per cent corn sirup plus 50 per cent sucrose (mixed
    cold before adding)
5. 50 per cent corn sirup plus 50 per cent sucrose (mixed
    as added)

These peaches were well ripened and had an excellent flavor when
packed. The fruit was judged after 6 months of storage.

The 100 per cent corn sirup packs had the best color, followed by 100
per cent sucrose, and 50 per cent corn sirup plus 50 per cent sucrose com-
binations. The boiled sirup combinations were the least desirable. The
natural fruit color increased with the increase in sugar concentrations
throughout the pack.

The body and texture of the peaches became less desirable with a decrease
in the sugar concentrations, except the (1 + 1) pack, in which the fruit was
pulped and the sugar was crystallized.

The best-flavored groups were the packs containing 50 per cent sucrose
plus 50 per cent corn sirup, mixed before adding, followed by the packs pre-
pared with 100 per cent corn sirup, 100 per cent sucrose, and 50 per cent
corn sirup and 50 per cent sucrose mixed as added. The boiled sirup sam-
ples were the least desirable. The intensity of the fruit flavor increased
with the increased sugar concentrations. The best individual sample was

the $(1+1)$ pack using 100 per cent corn sirup, followed by the $(2+1)$ concentration prepared with 100 per cent sucrose, and the sample containing 100 per cent corn sirup in a $(3+1)$ pack.

The same results were obtained using the same types of packs with a J. H. Hale and Gage-K50.

Elberta × Southhaven

These peaches were packed in 1-gallon paper containers using fruit-sugar concentrations of $(2+1)$, $(3+1)$, and $(4+1)$. The sweetening agents used were 80 per cent sucrose sirup, 80 per cent corn sirup, and 70 per cent corn sirup.

The fruit was well ripened when packed and had an excellent flavor.

The different packs all were judged to be excellent in color with no distinct difference in appearance. All samples also rated excellent in flavor, with the $(2+1)$ pack using 80 per cent sucrose sirup best, the $(2+1)$ 70 per cent corn sirup pack second, and the $(3+1)$ 80 per cent corn sirup pack last.

J. H. Hale × Elberta

This variety of peach was packed using fruit-sugar concentrations of $(2+1)$, $(3+1)$, and $(4+1)$. The sugars used in this pack were added in the form of sirup or on a dry basis in the combinations given below:

1. 100 per cent sucrose
2. 100 per cent corn sirup
3. 50 per cent corn sirup plus 50 per cent sucrose
4. 40 per cent corn sirup
5. 50 per cent corn sirup
6. 60 per cent corn sirup
7. 40 per cent sucrose sirup
8. 50 per cent sucrose sirup
9. 60 per cent sucrose sirup
10. 40 per cent sirup—half sugar and half corn sirup
11. 50 per cent sirup—half sugar and half corn sirup
12. 70 per cent sirup—57 per cent sugar and 43 per cent corn sirup

These peaches were ripe and had a good flavor when packed. The fruit was judged after 4 months of storage.

The $(2+1)$, $(3+1)$, and $(4+1)$ packs were placed in that order when judged for color. The best colors were evident when sirups of high sugar concentrations were used. Of the lots containing a single sweetening agent, the corn sirup pack was considered to have a better color than the sucrose pack. In the packs containing sucrose plus corn sirup combinations, there was little difference in the color of the various samples.

The best-flavored samples were the (2+1) packs, followed by (3+1) and (4+1) packs. The all-sucrose packs had the best flavor, followed by the 50 per cent sucrose plus 50 per cent corn sirup packs, and the all-corn sirup packs. The flavors of the peaches packed with different concentrations of sirups improved with the increase of total solids in the sirup used.

DISCUSSION

Of the varieties of peaches used in this study, the Gage crossed with Elberta proved to be most satisfactory from the standpoint of flavor, color, and body.

In the selection of a peach to be frozen-packed for later use in ice cream, it was found to be important that the fruit be well ripened, firm yet juicy, of golden yellow color and of distinct flavor. High sugar concentrations did not cause as excessive bleeding of the peach as it does in the case of fruits such as the strawberry. The best flavor usually resulted from the use of a high sugar concentration of two parts fruit and one part of sugar (2+1); however, satisfactory results were obtained with (3+1) packs. Packs containing only 20 per cent sugar (4+1) were not satisfactory because of the loss in flavor during storage.

Dextrose, because of its low solubility, crystallized when used alone. In combination with sucrose or corn sirup, little crystallization occurred in (3+1) packs when the proportion of dextrose was not greater than 50 per cent.

Corn sirup produced satisfactory results when used to replace 50 per cent of the sucrose. Such combinations were in some cases superior in flavor to the all-sucrose packs. When the corn sirup was used to replace all the sucrose, the flavor usually was considered less desirable.

Better fruit color resulted from the use of a combination of corn sirup and sucrose than when sucrose was used alone.

In comparing the different methods of adding the sugar and sirup to the fruit, it was found that adding the sugar in dry form gave best results. When sugar and corn sirup both were used, adding the fruit, sugar, and sirup in alternate layers was most satisfactory. Inverting of the containers at least once before final storage was found to aid in uniform mixing of the sugar and sirup with the fruit.

CONCLUSIONS

1. Peaches vary a great deal from the standpoint of their desirability for storage in frozen form.

2. Peaches to be frozen-packed should be well ripened, firm, juicy and of a golden yellow color.

3. For best flavor and color, the proportion of fruit to sweetening agent should be (2+1).

4. From the standpoint of flavor and color, a combination of 50 per cent cane sugar and 50 per cent corn sirup proved to be most satisfactory.

5. Because of its low solubility, dextrose should not be used in greater proportions than 50 per cent of the sweetening agent used and should not be used in fruit-sugar ratios in which the proportion of fruit to sugar is less than 3 to 1.

6. When using a combination of sugar and corn sirup, the fruit, sugar, and sirup should be added in alternate layers. There is no particular advantage in combining the sugar and sirup before adding to the fruit.

REFERENCE

(1) TRACY, P. H., RAMSEY, R. J., and RUEHE, H. A.   A Study of the Causes of a Stale Metallic Flavor in Strawberry Ice Cream.   Ill. Agr. Expt. Sta. Bul. 407.   1934.

HERITABILITY OF HEAT TOLERANCE IN DAIRY CATTLE

D. M. SEATH[1]

Louisiana Agricultural Experiment Station, Baton Rouge

Dairy cattle with a high degree of tolerance to heat are especially desirable in southern regions of the United States. Those possessing this heat-tolerance characteristic would be in special demand if they transmitted portions of it to their offspring. Otherwise, little permanent gain would be made in selecting animals possessing a high tolerance to heat.

Previous studies by Freeborn *et al.* (2) and Seath and Miller (9) have shown that Jerseys appear to be more tolerant to heat than do Holsteins. No attempt was made, however, to measure the degree to which heat tolerance is heritable. The present study was undertaken in an attempt to answer that question. It also was conducted in an effort to determine, if possible, what particular type of observation and how many observations should be made in order to best measure heat tolerance in dairy cattle.

MATERIAL AND METHODS

A description of the cows used and the method of securing the data have been covered in a previous paper (8). In brief, the procedure involved taking body (rectal) temperatures and respiration rates (from flank movements) of milking cows soon after they entered the milking barn at appproximately 3:00 p.m. In 1944, records were taken twice weekly over a period of 13 weeks between July 28 and October 24. During 1945, fifteen observations were made between July 16 and August 24.

The cows were handled in two separate dairy units, thus necessitating observations on separate days. Each unit consisted of both Jersey and Holstein cows. In 1944 there were 36 Holsteins and 16 Jerseys, while in 1945 there were 41 Holsteins and 27 Jerseys. Only 13 Holsteins and 8 Jerseys were the same for the 2 years.

RESULTS

Sire progeny rank and repeatability for two years. Body temperature and respiration rate averages for sire progeny groups (table 1) give some evidence that data taken duing one year are a reasonably good indication of what can be expected another year from groups of cows by the same sire.

137

In 1944 records were averaged on 52 daughters of 7 sires, the number of daughters varying from 4 to 11 per sire. During 1945 the number of daughters by these same 7 sires, plus one new one, totaled 68 and varied from 4 to 17 per sire with only 21 of them being the same as those observed in 1944.

The relative ranks of the respective sire progeny groups are much the same for the 2 years, with body temperature ratings more nearly alike than those for respiration rate. Of special interest are the high ratings on the basis of average body temperature for Holstein groups, with Jersey groups sharing the top ratings on the basis of respiration rate. It will be noted that daughters of Jersey sire no. 7 ranked highest in respiration rate for each of the 2 years, with over 100 respirations per minute. In contrast, the

TABLE 1

Body temperature and respiration rate averages and rank of sire progeny groups
(Progeny of 7 sires, using data on 8 warmer days)*

Sire no.	Breed	Body temperature				Respiration rate per minute			
		Av.		Relative rank		Av.		Relative rank	
		1944	1945	1944	1945	1944	1945	1944	1945
1	Holstein	104.27	104.26	2	2	79.0	81.5	7	5
2	"	104.19	103.91	3	3	81.6	86.3	3	4
3	"	103.79	103.89	4	5	88.2	94.2	2	2
4	"	104.40	104.26	1	1	79.2	80.0	6	7
5	Jersey	103.18	103.16	5	7	79.5	80.7	4	6
6	"	102.91	103.90	7	4	79.3	92.9	5	3
7	"	103.09	103.51	6	6	100.2	100.9	1	1

* Air temperature averaged approximately 89° F. for 8 days observed.

daughters of this same sire ranked sixth for each of the 2 years on the basis of body temperature. In reverse order, the progeny of Holstein sire no. 4 ranked highest in body temperature for both years; yet, on the basis of respiration rate, they ranked sixth in 1944 and seventh in 1945. Likewise, the progeny of sire no. 1 ranked second highest in body temperature each year, yet were seventh and fifth, respectively, in respiration rate for the 2 years.

Correlation coefficients between the average records for the 8 warm days in 1944 with those for 1945 were computed for the 21 cows common to the study for both years. Results of this study, when considered on an intra-breed-herd basis, yielded r values of 0.37 for body temperature and 0.64 for respiration rate. This shows that cows tend to react to warm weather in a given year similarly to the way in which they reacted the previous year.

The use of components of variance in the analysis. Analysis of variance (10) was used to segregate differences (a) between herds (included differences between days on which herds were observed), (b) between breeds within herds, (c) between sires within same breed and herd, (d) between

cows within same sire, breed, and herd group, and (e) between records of the same cow. An example of this method of segregating portions of the total variance can be seen in table 2. The procedure used in deriving an estimate of heritability of heat tolerance and the repeatability between single records of the same cow also is shown. This procedure involved, in principle, the computing of intra-class correlations (10)—in one case that between single records of paternal sisters, which was multiplied by 4 in order to secure an estimate of heritability of differences between single records (4), and in the second case that between single records of the same cow, which gave an estimate of average repeatability of single records (5).

TABLE 2

Analysis of variance for body temperature
(Data for milking cows observed on 8 warmer days in 1944)

Source of variance	d.f.	Mean square	Composition of mean square
Between herds	1	7.53	....
Between breeds within herds	2	45.65	....
Between sires within herd and breed	10	2.296	$E + cC + sS$
Between cows within sire, herd, and breed	38	1.43	$E + cC$
Between records of same cow	364	0.688	E

E = Variance between records of same cow = 0.688

c = Number of records per cow = 8

s = Computed number of records per sire group (4) = 28.32

C = Variance between cows within same sire, breed, and herd =

$$\frac{(E + cC) - E}{c} = \frac{1.43 - 0.688}{8} = 0.0927$$

S = Variance between sires within same breed and herd =

$$\frac{(E + cC + sS) - (E + cC)}{s} = \frac{2.296 - 1.43}{28.32} = 0.0306$$

Repeatability between single records of the same cow =

$$\frac{C + S}{E + C + S} = \frac{0.0927 + .0306}{0.688 + 0.0927 + 0.0306} = 0.152$$

Estimate of heritable portion of the variance =

$$\frac{4S}{E + C + S} = \frac{(4) \ 0.0306}{0.688 + 0.0927 + 0.0306} = 0.151$$

Days selected for measuring heat tolerance. A preliminary study was made to determine whether data for all test periods should be used for each year as a measurement of heat tolerance or whether data showing reactions of cows during the warmer days only would give the most accurate information. To determine this answer it was necessary to compare the repeatability of individual cow records when all data were used to that computed using the 8 warmer days, as shown for 1944 in table 2. These comparisons, as given in table 3, show in all cases that body temperature and respiration reactions tended to repeat themselves more closely on the 8 warm days than during the entire test periods. It is probable that this took place because

TABLE 3

Comparison of repeatability of single records of same cow for 8 warmer days versus entire period of test

	Using entire test period		Using 8 warmer days	
	1944	1945	1944	1945
Air temperatures for test periods				
Range	65–93	75–91	86–93	87–91
Average	85	86	89	89
Repeatability of single records				
Body temperature	0.080	0.067	0.152	0.385
Respiration rate	0.254	0.167	0.42	0.478

of the threshold effect, which causes cows to react differently and more as individuals when air temperatures rise above a certain level.

Data for the 8 warmer days also were the most useful in estimating the degree to which heat tolerance is heritable. As with repeatability between single records, it was found that the higher air temperatures as they existed on the 8 warmer days tended to increase the portion of the variance attributable to differences in heredity by from two to four times that found when data for all observation days were considered.

Heritability of body temperature changes. Analysis of the 1944 data for body temperature taken on the 8 warmer days is presented in table 2. Data taken in 1945 have been subjected to the same analysis. Results for the two years are shown in table 4. In general, it appears that the year 1945 produced greater variations that were attributable to differences between cows. As evidence of this, the repeatability between single records of the same cow was 0.385 or 38.5 per cent for 1945 as compared to 15.2 per cent for 1944.

Repeatability and heritability of respiration rate. When the analysis of variance procedure was applied to the respiration data taken on the 8 warmer days, the results (table 5) gave an estimate of repeatability. As shown in table 5, the repeatability in 1945 between records of the same cow was 0.481 or 48.1 per cent. The corresponding value for 1944 was 42 per

TABLE 4

Two-year comparisons of portions of variance concerned with heritability of body temperature

	1944	1945
C = Variance between cows of same sire, breed and herd	0.0927	0.2030
S = Variance between sires within same breed and herd	0.0306	0.0508
$\dfrac{C+S}{E+C+S}$ = Repeatability between single records of same cow	0.152	0.385
$\dfrac{4S}{E+C+S}$ = Estimate of heritable portion of variance	0.151	0.309

cent. The estimates of heritable portions of variance were surprisingly high, *i.e.*, 84.3 per cent for 1945 and 76.6 for 1944.

TABLE 5

Analysis of variance for respiration rate
(Data for milking cows observed on 8 warmer days in 1945)

Source of variance	d.f.	Mean square	Composition of mean square
Between herds	1	123.0	
Between breeds within herds	2	504.5	
Between sires within herd and breed	12	2444.5	$E + cC + sS$
Between cows within sire, herd and breed	52	743.8	$E + cC$
Between records of same cow	473	143.8	E

E = Variance between records of same cow = 143.8
c = Number records per cow = 8
s = Computed number records per sire group = 29.12
C = Variance between cows within same sire, breed, and herd = 75.00
S = Variance between sires within same breed and herd = 58.40
Repeatability between single records of the same cow = 0.481
Estimate of heritable portion of the variance = 0.843

DISCUSSION

Similarity between the ratings in 1944 and 1945 of seven sires on the basis of the response of their daughters to warm weather was quite striking (table 1). These ratings, on the bases of both body temperature and respiration rate, were enough alike for the 2 years to suggest that inheritance must play an important part in causing differences to exist in heat tolerance among dairy cows. Discrepancy between the ratings on the basis of respiration rate as compared to those for body temperature, however, leaves doubt as to the emphasis that should be placed on each of the two heat-tolerance measurements. As was pointed out, the progeny of certain sires ranked near the top in respiration rate, yet near the bottom in body temperature, with the reverse true in one or two cases. This took place even though studies show (3, 8) that both body temperature and respiration rate are correlated closely with air temperature. It would seem that body temperature is probably the safer index on which to judge heat tolerance, since this is no good reason for wanting cows to have high body temperatures. On the other hand, the inheritance of fast breathing by a cow may aid her considerably in quickly eliminating excess heat, which contributes to her comfort (and possibly her health) by more nearly maintaining a normal body temperature.

Repeatability between the averages of warm-weather records for consecutive years among cows of the same breed, based on only 21 cows common to the study for the 2 years, was highest for respiration ($r = 0.64$) and lowest for body temperature ($r = 0.37$). In the case of both respiration and body temperature, these results show that the cow as an individual reacts to warm weather in a manner which is similar from year to year and suggests

that the reason for the similarity in reactions must be due, at least partially, to inheritance.

Results of this study are in line with findings by Regan and Freeborn (7) and the general practice of making heat-tolerance observations on warm days only. When 8 warm days were used, the individual records of each cow show repeatability averages for body temperature of 15.2 per cent and 38.5 per cent, respectively, for the 2 years. For respiration rate the repeatability was higher, being 42 per cent and 47.8 per cent, respectively.

Efforts to estimate heritability of individual body temperature records on the basis of sire-progeny differences, although subject to much sampling error, gave results which appear reasonable, i.e., 15.1 per cent in 1944 and 30.9 per cent in 1945. These are approximately the same as the 15.2 per cent and 38.5 per cent which represent the estimates for repeatability of individual records of the same cow for these 2 years. In general, one would expect the repeatability percentage to exceed that for heritability (5), for a cow tends to repeat her performance, not only because of her specific inheritance but also because of certain factors peculiar to herself, including those involved in her environment.

If 15 to 30 per cent is a reasonable estimate of heritability, then one can closely predict what progress can be made through breeding toward more tolerance to high air temperature. For example, if selection in a herd results in saving, as parents, cows (and a bull) that average 1° F. lower in body temperature (when tested on a warm day) than the average of the entire herd, then one would expect offspring from these selected parents to average from 0.15 to 0.3° F. lower than the herd average when subjected to a similar test. This degree of heritability is in line with that found when considering single production records of dairy cows (5, 6). In both cases, as explained by Lush (5), the use of more than one record increases the heritable portion of the variance, although it does lower slightly the spread between the average of those saved and the average for the herd, which is spoken of as the selection differential. Even so, the increase is worthwhile. Using two records with an average heritability of 20 per cent would increase progress from selection by 29 per cent over that secured when only one record is used. The increase would be 58 per cent using four records, and 83 per cent when eight records are used.

Mention already has been made of the unexpected results secured from a study of respiration rates. The estimates for heritability of 76.6 per cent for 1944 and 84.3 per cent for 1945 greatly exceed those for repeatability of single records, which were 42 per cent and 48.1 per cent, respectively, for these 2 years. In general, one can expect repeatability percentages to exceed those for heritability for reasons already explained in connection with body temperature comparisons. Cases where this does not occur can be explained by sampling errors, e.g., calculation of fiducial

limits for heritability[2] indicates that results secured are well within the range of expectation, considering number of animals tested and number of sires involved in the estimates.

In general, this experiment has yielded results which indicate that respiration rate as a measure of tolerance to heat, while highly repeatable, gives results which are hard to explain and does not appear to coincide closely enough with body temperature as a measurement to permit general use of respiration rate, to the exclusion of other tests. On the other hand, body temperature, while slightly less repeatable, does appear to be a good test for heat tolerance and yields results which are reasonable.

SUMMARY

Tests of Jerseys and Holsteins involving 52 cows by 7 sires in 1944 and 68 cows by 8 sires in 1945 with respect to the heritability of heat tolerance as indicated by variations in body temperature and respiration rate gave results as follows:

1. Ranking of sire progeny by years showed a great similarity for the 2 years, although there was discrepancy between rank on basis of body temperature and that for respiration rate. Some sire groups ranked high on one basis and low on the other and vice versa.

2. Twenty-one cows included in study for both years showed correlations between average records for 8 warmer days (on an intra-herd-breed basis) of 0.37 for body temperature and 0.64 for respiration rate.

3. Using records for 8 warmer days gave a repeatability for individual body temperature records of same cow of 15.2 per cent for 1944 and 38.5 per cent for 1945, as compared to 8 per cent and 6.7 per cent for the 2 years when all observation days were used. In like manner, respiration rates were more highly repeatable using only the warmer days.

4. Estimates of heritability of individual records based on sire-progeny differences were for body temperature 15.1 per cent and 30.9 per cent for the 2 years, and for respiration 76.6 per cent and 84.3 per cent. Figures for respiration appear out of line, as they greatly exceed the estimates of repeatability and the reverse condition was expected.

5. Body temperature appears to be a safer measuring stick for heat tolerance than does respiration rate.

6. The estimate of heritability of body temperature (15 to 30 per cent) is in line with that found for individual production records of cows. In practice this would mean that the offspring from parents selected because of their tolerance to heat would be expected to retain from 15 to 30 per cent of the advantage that the parents had over the average for the herd or breed.

[2] Fiducial limits at the 5 per cent level of significance computed as per methods outlined by Fisher (1) resulted in estimates of heritability of single respiration records ranging from 22 to 177 per cent in 1944 and from 25.6 to 232 per cent in 1945.

7. Using more than one record greatly increases progress through selection. If heritable portion of variance between single heat tolerance record is 20 per cent, then progress through selection would increase by 29 per cent using two records, 58 per cent with four, and 83 per cent using eight records.

REFERENCES

(1) FISHER, R. A. Statistical Methods for Research Workers. 7th Ed. Oliver and Boyd., London. pp. 236–240. 1938.

(2) FREEBORN, STANLEY B., REGAN, WILLIAM M., AND BERRY, LESTER J. The Effect of Petroleum Oil Sprays on Dairy Cows. Jour. Econ. Ent., 27: 382–388. 1934.

(3) GAALAAS, R. F. Effect of Atmospheric Temperature on Body Temperature and Respiration Rate of Jersey Cattle. Jour. Dairy Sci., 28: 555–563. 1945.

(4) HETZER, H. O., DICKERSON, G. E., AND ZELLER, J. H. Heritability of Type in Poland-China Swine as Evaluated by Scoring. Jour. Anim. Sci., 3: 393–394. 1944.

(5) LUSH, J. L. Animal Breeding Plans. 3rd Ed. The Iowa State College Press, Ames, Iowa. pp. 170–179. 1945.

(6) LUSH, J. L., AND STRAUS, F. S. The Heritability of Butterfat Production in Dairy Cattle. Jour. Dairy Sci., 25: 975–982. 1942.

(7) REGAN, W. M., AND FREEBORN, S. B. The Effect of Flies and Fly Sprays on Certain Physiological Processes of the Dairy Cows. Jour. Dairy Sci., 19: 11–28. 1936.

(8) SEATH, D. M., AND MILLER, G. D. The Relative Importance of High Temperature and High Humidity as Factors Influencing Respiration Rate, Body Temperature, and Pulse Rate of Dairy Cows. Jour. Dairy Sci., 29: 465–472. 1946.

(9) SEATH, D. M., AND MILLER, G. D. Heat Tolerance Comparisons Between Jersey and Holstein Cows. Jour. Anim. Sci. (in press). 1946.

(10) SNEDECOR, G. W. Statistical Methods. 4th Ed. The Iowa State College Press. Ames, Iowa. pp. 214–264. 1946.

A COMPARISON OF THE BABCOCK, GERBER, MINNESOTA, PENNSYLVANIA, AND MOJONNIER METHODS FOR DETERMINING THE PERCENTAGE OF FAT IN HOMOGENIZED MILK[1]

G. M. TROUT AND P. S. LUCAS

Michigan Agricultural Experiment Station, East Lansing, Mich.

The general acceptance of homogenized milk today has made imperative a study of the existing methods for testing it for butterfat. Extensive studies have been made by several workers on the various methods of testing nonhomogenized milk for butterfat, but the data are somewhat limited when the methods are applied to homogenized milk.

LITERATURE

The Roese-Gottlieb (Mojonnier) Method

Burr (6), comparing several methods for testing homogenized milk for fat, found the Roese-Gottlieb method the most accurate. This test was so considered by all the chemists at that time. Richmond (28) pointed out that for ease and accuracy the Roese-Gottlieb method appeared to be the best method for determining the percentage of fat in homogenized milk. Marquardt (23) stated that ether extraction methods gave the most reliable results when testing homogenized milk for fat. Doan (12) stated that the homogenization process did not influence the accuracy of the Roese-Gottlieb or the Mojonnier methods. Mojonnier (25) reported the results of nine tests made by the Kohler and Kohler Laboratories showing that homogenized milk tested by the Mojonnier method averaged 0.012 per cent less than the same milk not homogenized. Dahlberg, Holm, and Troy (11), in comparing the Roese-Gottlieb tests of milk made by five different laboratories, double homogenized one sample at 2,500 lbs. pressure to render it homogeneous for all laboratories. The sample of homogenized milk did not yield tests with the least variation between duplicates or between laboratories.

The Babcock Method

The literature on the Babcock method for testing homogenized milk has been reviewed by Herreid (15) and by Trout and Lucas (34) and need not be repeated in full here. However, some review seems necessary to point out the relationship of the procedure in question with the Mojonnier method.

Nonhomogenized milk. Dahlberg (9) showed, in a comparison of 32 tests of nonhomogenized milk varying in fat content from 4.42 to 4.92 per cent, the Babcock test to be an average of 0.1 per cent high, reading from

the bottom of the lower meniscus to the extreme top of the upper meniscus. Phillips (27) on 50 trials observed that the Babcock tests of nonhomogenized milk ranged from 0.005 to 0.126 and averaged 0.0588 per cent higher than the Roese-Gottlieb tests. Wilster and Robichaux (36) found in testing 1,380 samples that the Babcock test of nonhomogenized milk ranged from 0.074 to 0.077 per cent higher than the Mojonnier tests. They reported no studies on homogenized milk samples.

Hileman *et al.* (18), surveying the literature, reported that twelve of fourteen investigators secured results with the Babcock test on nonhomogenized milk slightly higher than the Mojonnier, the over-all average being + 0.076 per cent above that of the Mojonnier. Fahl, Lucas, and Baten (13) found the Babcock tests higher than those for the Mojonnier. Herreid *et al.* (16), in accounting for milk fat, secured results by the Mojonnier method on unpreserved milk which agreed with those obtained by the Babcock method.

On the other hand, some investigators reported lower tests by the Babcock method than by the Mojonnier. Dahlberg (9), although securing higher results by the Babcock method when the fat column was read according to standard technic, nevertheless did secure lower tests when oil was applied to destroy the meniscus. Dahlberg, Holm, and Troy (11) did not verify the results of several previous investigations which tended to show that the Babcock test gave slightly but uniformly higher percentages of fat than the Mojonnier.

Mojonnier and Troy (24) reported data showing that the difference between the Babcock and Mojonnier tests was not constant in one direction but that the Babcock test varied both above and below that of the Mojonnier. Later, Mojonnier (25) reported the results of Babcock tests on nine samples of homogenized milk which averaged 0.072 per cent higher than the Mojonnier tests. Other tests indicated a higher percentage of fat in the fat columns of test of homogenized milk than in those on nonhomogenized milk.

From calculations of the data presented by Babcock (3) in introducing his test, of 30 samples of whole milk tested by the Babcock and gravimetric methods, 15 Babcock tests were above and 15 were below those of the ether-extraction method. The Babcock test results varied from − 0.16 to + 0.30 per cent from results of the gravimetric procedure. The average tests differed by 0.01 per cent, the Babcock method being higher.

Homogenized milk. Generally the Babcock test of homogenized milk yields results slightly lower than on the same milk not homogenized, being within the 0.1 per cent tolerance for the test. Workers of the Arizona Experiment Station (1) reported comparison of some 30 samples of homogenized milk from different sources, and homogenized at different pressures, indicated that the Mojonnier test was from 11 to 25 per cent higher than the Babcock test. Later (2) they reported data on six trials of milk homogen-

ized from 2,000 to 4,000 lbs. pressure which showed that the Mojonnier test consistently gave results higher than the Babcock and that the difference was believed sufficient to justify a correction when the Babcock method was used for homogenized milk.

Doan (12), using a modified Babcock method, secured results from 0.084 to 0.11 per cent higher than those by the Mojonnier method, the differences being only insignificantly higher than the average in the literature for non-homogenized milk. Lampert and Brandon (21) secured results on homogenized milk within an average of +0.03 per cent of the Mojonnier results when using the regular Babcock method on unpreserved samples. This was within 0.01 per cent of the Babcock tests on nonhomogenized milk. Homogenized samples preserved with formalin averaged 0.13 per cent less than by the extraction method. They concluded that the regular Babcock procedure gave accurate results on unpreserved homogenized milk, but a lower test when the sample was preserved with formaldehyde. Trout and Lucas (34) reported that many modifications of the Babcock method existed. Averages of results of five trials on each of eleven modified Babcock tests varied from those of the Mojonnier test from − 0.03 to − 0.15 per cent, depending chiefly upon the modification.

Webster (35) observed in four trials on homogenized milk that the Babcock test averaged 0.088 per cent lower than the Mojonnier test. He reported that the variation between the Babcock and Mojonnier methods was affected directly by the size of the fat globules or, more particularly, by the relative number of small fat globules present. He concluded that the statement that the Babcock test over-reads the Mojonnier or gravimetric method by some stated percentage seems of little value unless the size of the fat globules is considered.

The Gerber Method

Nonhomogenized milk. Fisher and Walts (14) found the average variation from the Roese-Gottlieb was ± 0.137 per cent for the Babcock and ± 0.122 per cent for the Gerber method in testing nonhomogenized milk. Both the Babcock and the Gerber tests for milk in 11 instances (68.75 per cent) gave results which were slightly higher than the Roese-Gottlieb. They believed there was no advantage in introducing to the industry another method, the Gerber, which was not more accurate than the Babcock method. Dahlberg, Holm, and Troy (11) and Dahlberg (10), applying the test to nonhomogenized milk, found that the Gerber and Babcock tests were comparable from the standpoint of accuracy but recommended that one good practical test for fat in milk was better than two of equal merit.

Van der Burg (5) compared the Gerber with other fat tests, including the Roese-Gottlieb, on 85 samples of milk. Generally the Gerber gave slightly higher results than the other methods. In 12 samples the Gerber fat values were identical with other values, in 31 samples they were 0.005

to 0.025 per cent higher, and in 24 samples 0.005 to 0.025 per cent lower. Fourteen samples varied ± 0.030 to ± 0.050 per cent and 4 samples ± 0.055 to ± 0.100 per cent.

Homogenized milk. Buttenberg (8) pointed out that the determination of the fat content of homogenized milk by the Adams method, commonly used at that time, could not show results comparable with the Roese-Gottlieb method; even when the extraction was prolonged purposely from 10 to 12 hours, the results were much too low.

Siegfeld (29) used the Gerber method for testing homogenized milk with excellent results, but pointed out that it was necessary in testing homogenized milk by this method to centrifuge 12 minutes instead of the customary 3 minutes. Burr (6), comparing several methods, found that the Gerber method, while fairly satisfactory, gave results which varied with the time of centrifuging. The homogenized milk examined was processed at 60 atmospheres (approximately 900 lbs.) pressure. Average results of 11 trials showed the Gerber test centrifuged 10 minutes varied from the Roese-Gottlieb by ± 0.02 per cent, − 0.08 to + 0.125 per cent being the range of variation.

Richmond (28) found the Gerber test gave good results with homogenized milk, but that "the advent of homogenized milk rendered it necessary to remove the Adams method from the position it had so long occupied as a standard method." Six trials showed that the Gerber test varied + 0.008 per cent from the Roese-Gottlieb tests of the same milk. Istaz and Van Soest (20) observed that the results secured on homogenized milk by the Gerber method were verified by the gravimetric method in many cases.

Hoyberg (19) had difficulty securing results by the Gerber method comparable with those of the Roese-Gottlieb method, even when centrifuging as long as 45 to 60 minutes, or when prolonging the holding time in the water bath after centrifuging. By heating the milk to 60–65° C., holding it 5 minutes and then making the Gerber test, results identical with those obtained by the Roese-Gottlieb method were secured. He advised pouring heated milk directly into the sulfuric acid and amyl alcohol mixture rather than letting it run down the side of the butyrometer. This resulted in an increased amount of heat liberation, which was deemed important in testing homogenized milk. Milk heated to 15° C. increased to 75° C. during its reaction with the sulfuric acid and amyl alcohol. At 25° C. it increased to 82°, at 40° to 86°, and at 45° to 88° C. Milk heated to 60–65° C. would reach a temperature of 105° C. when added to the acid.

Burr and Weise (7) found that the Gerber method gave comparable 'but slightly higher fat tests of homogenized milk than the Roese-Gottlieb method. However, in using the Gerber test, double centrifuging was necessary. In 18 trials the Gerber method gave results 0.03 per cent higher than the Roese-Gottlieb method. Von Sobbe (30) found the Gerber method very

satisfactory for testing homogenized milk, but noted that centrifuging had to be repeated at least twice, and that the mixture of sulfuric acid and milk plasma had to be clear and transparent. In making the test he warmed the milk to 60–65° C., then cooled it with agitation to remove the disturbing effects of homogenization. Herrington (17) pointed out that the amyl alcohol used in the Gerber test was a possible source of error in the test. The error caused by the test might be 0.1–0.2 per cent or even higher. He recommended that boiling between 128° C. and 132° C. was the best method of identifying any sample of amyl alcohol to be used in making the Gerber test.

The Minnesota-Babcock Method

While the Minnesota-Babcock test is used for the fat determination of all dairy products (26), few data were found in the literature on its use in testing homogenized milk. Trout, Halloran, and Gould (33) and Trout (32) studied the possibilities of the Minnesota reagent for overcoming char formation in testing homogenized milk. They reported results comparable in appearance with the best results obtained by the Babcock method but presented no data comparing the accuracy of the two methods.

Lampert and Brandon (21) on two trials secured tests on homogenized milk by the Minnesota method which ranged from 0.22 to 0.26 per cent less than the Mojonnier tests. The samples were preserved with mercuric chloride. They stated, "Samples preserved with mercuric chloride reacted with the reagent so that a black precipitate, probably finely divided mercury, was formed. This material entered the fat column, but in a number of cases the fat column dropped through this material, leaving it adhering to the neck of the test bottle. Although the fat tests appeared excellent, the results were lower than those obtained with the other procedures (Mojonnier, Babcock, and Pennsylvania), and the use of the Minnesota test was not continued." They concluded that the method could not be recommended for the testing of homogenized milk. Bird and Breazeale (4) observed wide variations in the fat content of the same sample of buttermilk tested by three Minnesota reagents.

The Pennsylvania Method

Few data were found on the use of the Pennsylvania method for testing homogenized milk. Swope (31) recommended it for the testing of homogenized milk. He reported several tests on two samples of homogenized milk, one sample of which showed an average arithmetical deviation from the Mojonnier test of 0.036 per cent and an average algebraic deviation + 0.024 per cent. The second sample showed an arithmetical deviation of 0.047 per cent and an average algebraic deviation of + 0.041 per cent. The range in deviation of the Pennsylvania tests from the Mojonnier tests was from − 0.009 to + 0.091 per cent.

Lampert and Brandon (21) found the Pennsylvania test yielded an average of 0.10 per cent less fat on the homogenized milk than on the same milk not homogenized, the tests of the nonhomogenized and homogenized milk being + 0.22 and + 0.17 per cent higher, respectively, than those obtained by the Mojonnier method. Difficulty was encountered in making satisfactory tests of homogenized milk to which preservatives had been added. They concluded that the Pennsylvania test could not be recommended for the testing of homogenized milk.

SCOPE OF THE INVESTIGATION

Inasmuch as homogenized milk is of increasing importance in market milk distribution, a comparative study of the various more common methods of testing milk for fat seemed advisable. In this investigation the following methods were compared: Mojonnier, modified Babcock, Gerber, Minnesota-Babcock, Pennsylvania, and a modified Pennsylvania method. Data were secured from duplicate tests on 24 samples for each of the above methods. These tests were not necessarily designed for testing homogenized milk. However, it seemed desirable to include all of them in the study since some of them (a) are more or less common tests in testing laboratories, (b) are used in testing milk in vocational high schools, (c) employ chemicals other than sulfuric acid, which might thus prevent char formation, (d) are readily available, (e) are moderately priced, and (f) are not too difficult for routine analyses.

PROCEDURE

The milk tested was that regularly processed in the College Creamery. The nonhomogenized samples were taken from the vat after pasteurization and prior to homogenization. The milk had been kept thoroughly mixed during pasteurization and homogenization in order to insure uniform fat distribution. The homogenized samples were taken from the cooled bottled product after homogenization was well under way. Mojonnier tests showed that the nonhomogenized and homogenized samples contained similar percentages of fat.

Homogenization was done by means of a 500-gallon-per-hour viscolizer at 2,500 lbs. pressure at 130° to 140° F. following pasteurization. The collected samples were cooled adequately, stored and tested as rapidly as time would permit. Pipetting of the portions of milk into the test bottles was done for all tests at one time to assure correct sampling. This was done after the milk had been tempered at 70° F. for 2 hours. These charged test bottles were then stored at 40° F. until the tests were made.

The Mojonnier test was used as a standard for accuracy. Instead of using an approximately ten-gram portion measured volumetrically, duplicate samples of milk previously tempered at 70° F. were weighed carefully on a chemical balance directly into a fat-extraction flask.

The tests using Babcock test bottles were made in bottles which had been recalibrated for accuracy. Any bottles showing 0.1 per cent or more variation from exact accuracy were discarded. The modified Babcock procedure employed 17.5 ml. of 1.835 specific gravity sulfuric acid added in three portions, 8.0, 5.0 and 4.5 ml., respectively. Mixing was prolonged for at least 2 minutes after final addition of the acid, as suggested by Lucas and Trout (22); however, the water-alcohol solution was not added to support the fat column for reading. The Gerber (Fucoma) test was carried out according to the directions of the Fucoma Company. The reagents used in the Minnesota-Babcock method were from the Kimble Glass Co. The modification of the Pennsylvania test consisted in the use of sulfuric acid having a specific gravity of 1.81 instead of 1.73, as recommended and as used in the regular procedure.

RESULTS

The Mojonnier method. The data secured on testing nonhomogenized and homogenized milk by the Mojonnier method are presented in table 1.

TABLE 1

Mojonnier fat tests of nonhomogenized and homogenized milk

Series*	Mojonnier method						Variation from test of nonhomogenized milk
	Nonhomogenized milk			Homogenized milk			
	Duplicates		Av.	Duplicates		Av.	
	%	%		%	%		
1	3.651	3.650	3.65	3.672	3.671	3.67	+ 0.02
2	3.708	3.699	3.70	3.701	3.694	3.70	0.00
3	3.695	3.716	3.71	3.712	3.695	3.70	− 0.01
4	3.723	3.716	3.72	3.724	3.738	3.73	+ 0.01
5	3.814	3.801	3.81	3.826	3.817	3.82	+ 0.01
6	3.831	3.804	3.82	3.804	3.815	3.81	− 0.01
7	3.850	3.864	3.86	3.835	3.832	3.83	− 0.03
8	3.916	3.886	3.90	3.898	3.882	3.89	− 0.01
9	3.897	3.926	3.91	3.918	3.903	3.91	0.00
10	4.375	4.409	4.39	4.407	4.404	4.41	+ 0.02
11	4.470	4.476	4.47	4.493	4.503	4.50	+ 0.03
12	4.532	4.537	4.53	4.543	4.552	4.55	+ 0.02
13	4.585	4.575	4.58	4.560	4.570	4.57	− 0.01
14	4.556	4.592	4.57	4.594	4.593	4.59	+ 0.02
15	4.591	4.589	4.59	4.580	4.600	4.59	0.00
16	4.591	4.596	4.59	4.597	4.586	4.59	0.00
17	4.575	4.628	4.60	4.653	4.634	4.64	+ 0.04
18	4.619	4.606	4.61	4.608	4.606	4.61	0.00
19	4.606	4.627	4.62	4.641	4.644	4.64	+ 0.02
20	4.763	4.761	4.76	4.759	4.768	4.76	0.00
21	4.786	4.789	4.79	4.785	4.777	4.78	− 0.01
22	4.956	4.973	4.96	4.936	4.934	4.94	− 0.02
23	5.012	5.038	5.03	5.049	5.04	5.04	+ 0.01
24	5.124	5.082	5.10	5.04	5.08	5.06	− 0.04
Av.	....	....	4.344	....	....	4.347	0.0024

* Arranged according to increasing percentages of fat.

The average test was 4.344 per cent for nonhomogenized milk and 4.347 per cent for homogenized milk. Of the 24 samples, 6 tested exactly the same as the nonhomogenized; 8 tested lower, ranging from − 0.01 to − 0.04 per cent; and 10 tested higher, ranging from + 0.01 to + 0.04 per cent. Thus it appears that homogenized milk may be tested reliably by the Mojonnier method.

The modified Babcock method. The same samples of milk tested by the

TABLE 2
Comparison of modified Babcock and Mojonnier tests of homogenized milk

Series*	Modified Babcock method			Variations from Mojonnier	
	Nonhomo-genized	Homogenized	Variation of homogenized from non-homogenized	Nonhomo-genized	Homogenized
	%	%			
1	3.72	3.70	− 0.02	+ 0.07	+ 0.03
2	3.72	3.70	− 0.02	+ 0.02	0.00
3	3.77	3.75	− 0.02	+ 0.06	+ 0.05
4	3.78	3.78	0.00	+ 0.06	+ 0.05
5	3.90	3.88	− 0.02	+ 0.09	+ 0.06
6	3.85	3.85	0.00	+ 0.03	+ 0.04
7	3.90	3.88	− 0.02	+ 0.04	+ 0.05
8	3.98	3.93	− 0.05	+ 0.08	+ 0.04
9	3.95	3.93	− 0.02	+ 0.04	+ 0.02
10	4.48	4.48	0.00	+ 0.09	+ 0.07
11	4.53	4.53	0.00	+ 0.06	+ 0.03
12	4.58	4.58	0.00	+ 0.05	+ 0.03
13	4.63	4.63	0.00	+ 0.05	+ 0.06
14	4.67	4.60	− 0.07	+ 0.10	+ 0.01
15	4.65	4.63	− 0.02	+ 0.06	+ 0.04
16	4.63	4.63	0.00	+ 0.04	+ 0.04
17	4.67	4.65	− 0.02	+ 0.07	+ 0.01
18	4.70	4.70	0.00	+ 0.09	+ 0.09
19	4.68	4.70	+ 0.02	+ 0.06	+ 0.06
20	4.80	4.80	0.00	+ 0.04	+ 0.04
21	4.80	4.80	0.00	+ 0.01	+ 0.02
22	5.00	4.95	− 0.05	+ 0.04	+ 0.01
23	5.08	5.13	+ 0.05	+ 0.05	+ 0.09
24	5.17	5.15	− 0.02	+ 0.07	+ 0.09
Av.	4.402	4.390	− 0.012	+ 0.057†	+ 0.043†

* The tests reported in this table were included among the data on the 36 trials previously reported by Lucas and Trout (22).
† Highly significantly different from zero.

Mojonnier method also were tested by the modified Babcock method. The nonhomogenized and homogenized milk averaged 4.402 and 4.390 per cent butterfat, respectively (table 2). Of the 24 samples tested, 10 tests were identical with those of the nonhomogenized milk; 12 were lower, ranging from − 0.02 to − 0.07; and 2 were higher, + 0.02 and + 0.05. The nonhomogenized milk averaged 0.057 per cent higher by this method than by the Mojonnier; the homogenized averaged only 0.043 per cent higher.

The Gerber method. The average Gerber tests of the nonhomogenized and homogenized milk were practically identical, the average test on homogenized milk being 0.003 per cent higher (table 3). Of the 24 comparisons, 12 were the same; 5 of the homogenized were lower, ranging from – 0.02 to – 0.07 per cent; and 7 were higher, ranging from + 0.02 to + 0.08 per cent. The average tests of both the nonhomogenized and the homogenized averaged

TABLE 3

Comparison of Gerber and Mojonnier tests of homogenized milk

Series	Gerber method			Variations from Mojonnier	
	Nonhomogenized	Homogenized	Variation of homogenized from non-homogenized	Nonhomogenized	Homogenized
	%	%			
1	3.73	3.73	0.00	+ 0.08	+ 0.06
2	3.78	3.78	0.00	+ 0.08	+ 0.08
3	3.80	3.80	0.00	+ 0.09	+ 0.10
4	3.83	3.83	0.00	+ 0.11	+ 0.10
5	3.93	3.95	+ 0.02	+ 0.12	+ 0.13
6	3.85	3.87	+ 0.02	+ 0.03	+ 0.06
7	3.95	3.95	0.00	+ 0.09	+ 0.12
8	4.00	4.00	0.00	+ 0.10	+ 0.11
9	4.03	3.96	– 0.07	+ 0.12	+ 0.05
10	4.50	4.52	+ 0.02	+ 0.11	+ 0.11
11	4.60	4.58	– 0.02	+ 0.13	+ 0.08
12	4.60	4.62	+ 0.02	+ 0.07	+ 0.07
13	4.68	4.68	0.00	+ 0.10	+ 0.11
14	4.72	4.70	0.00	+ 0.15	+ 0.11
15	4.65	4.67	+ 0.02	+ 0.06	+ 0.08
16	4.70	4.70	0.00	+ 0.11	+ 0.11
17	4.68	4.73	+ 0.05	+ 0.08	+ 0.09
18	4.70	4.68	– 0.02	+ 0.09	+ 0.07
19	4.67	4.75	+ 0.08	+ 0.05	+ 0.11
20	4.85	4.83	– 0.02	+ 0.09	+ 0.07
21	4.88	4.88	0.00	+ 0.09	+ 0.10
22	5.00	4.98	– 0.02	+ 0.04	+ 0.04
23	5.10	5.10	0.00	+ 0.07	+ 0.06
24	5.20	5.20	0.00	+ 0.10	+ 0.14
Av.	4.434	4.437	+ 0.003	+ 0.090*	+ 0.090*

* Highly significantly different from zero.

0.09 per cent higher than the corresponding Mojonnier tests. In making the Gerber tests of homogenized milk the following factors were striking: (a) the clarity of the fat column and supporting liquid, (b) the identical reading of the duplicate tests, (c) the consistent check with tests on the nonhomogenized milk, and (d) the complete freedom of any char formation.

The Minnesota method. While the Minnesota-Babcock test of homogenized milk varied from that of the nonhomogenized milk by an average of only + 0.027 per cent, the range of variations between the tests extended from – 0.32 to + 0.40 per cent. Only 2 of the 24 tests were identical with those

of the nonhomogenized milk, while 12 were below, ranging from -0.02 to -0.30, and 10 were above, ranging from $+0.07$ to $+0.40$ per cent (table 4). Tests of both nonhomogenized and homogenized milk were consistently under the Mojonnier tests, the tests on the homogenized milk ranging from -0.04 to -0.85 and averaging -0.433 per cent.

The Pennsylvania method. The Pennsylvania tests on homogenized milk consistently were under those of the nonhomogenized milk, -0.07 to -0.72

TABLE 4

Comparison of Minnesota and Mojonnier tests of homogenized milk

Series	Minnesota method			Variations from Mojonnier	
	Nonhomo-genized	Homogenized	Variation of homogenized from non-homogenized	Nonhomo-genized	Homogenized
	%	%			
1	3.07	3.35	+ 0.28	− 0.58	− 0.32
2	3.08	2.88	− 0.20	− 0.62	− 0.82
3	3.05	3.35	+ 0.30	− 0.66	− 0.35
4	3.38	3.28	− 0.10	− 0.34	− 0.45
5	3.25	3.23	− 0.02	− 0.56	− 0.59
6	3.50	3.43	− 0.07	− 0.32	− 0.38
7	3.15	2.98	− 0.17	− 0.71	− 0.85
8	3.22	3.20	− 0.02	− 0.68	− 0.69
9	3.33	3.48	+ 0.15	− 0.58	− 0.43
10	4.00	4.07	+ 0.07	− 0.39	− 0.34
11	4.23	3.93	− 0.30	− 0.24	− 0.57
12	4.00	4.30	+ 0.30	− 0.53	− 0.25
13	4.08	4.40	+ 0.32	− 0.50	− 0.17
14	3.95	3.95	0.00	− 0.62	− 0.64
15	4.30	4.55	+ 0.25	− 0.29	− 0.04
16	4.23	4.35	+ 0.12	− 0.36	− 0.24
17	4.47	4.15	− 0.32	− 0.13	− 0.49
18	4.05	4.20	+ 0.15	− 0.55	− 0.41
19	4.20	3.98	− 0.22	− 0.42	− 0.66
20	4.57	4.45	− 0.12	− 0.19	− 0.31
21	4.20	4.60	+ 0.40	− 0.59	− 0.18
22	4.43	4.35	− 0.08	− 0.53	− 0.59
23	4.76	4.76	0.00	− 0.27	− 0.28
24	4.77	4.70	− 0.07	− 0.33	− 0.36
Av.	3.886	3.913	+ 0.027	− 0.458*	− 0.433*

* Highly significantly different from zero.

and averaging -0.538, and were under the Mojonnier readings in 18 of the 24 trials, or 75 per cent (table 5). The readings ranged from -0.31 to -0.50 per cent under those of the Mojonnier and from $+0.03$ to $+0.16$ per cent above, and averaged -0.29 per cent. Nevertheless, the same tests on the nonhomogenized milk consistently were above those of the Mojonnier, averaging $+0.255$ per cent higher.

a. The modified Pennsylvania method. Since the average Pennsylvania tests on the homogenized milk were lower than both the Pennsylvania test

and the Mojonnier test for nonhomogenized milk, attempts were made to improve the test for homogenized milk by increasing the specific gravity of the acid used from 1.73 to 1.81. This modification increased the reading on the nonhomogenized milk slightly, averaging about 0.02 per cent, and that of the homogenized milk about 0.53 per cent, bringing the average readings of the nonhomogenized and homogenized milk within 0.032 per cent of each

TABLE 5

Comparison of Pennsylvania and Mojonnier tests of homogenized milk

Series	Pennsylvania method			Variations from Mojonnier	
	Nonhomo-genized	Homogenized	Variation of homogenized from non-homogenized	Nonhomo-genized	Homogenized
	%	%			
1	3.88	3.28	− 0.60	+ 0.23	− 0.39
2	3.87	3.20	− 0.67	+ 0.17	− 0.50
3	4.05	3.83	− 0.22	+ 0.34	+ 0.13
4	4.05	3.88	− 0.17	+ 0.33	+ 0.15
5	4.15	3.85	− 0.30	+ 0.34	+ 0.03
6	4.03	3.38	− 0.65	+ 0.21	− 0.43
7	4.20	3.88	− 0.32	+ 0.34	+ 0.05
8	4.20	4.00	− 0.20	+ 0.30	+ 0.11
9	4.17	3.55	− 0.62	+ 0.26	− 0.36
10	4.65	3.98	− 0.67	+ 0.26	− 0.43
11	4.67	4.10	− 0.57	+ 0.20	− 0.40
12	4.80	4.08	− 0.72	+ 0.27	− 0.47
13	4.82	4.10	− 0.72	+ 0.24	− 0.47
14	4.82	4.20	− 0.62	+ 0.25	− 0.39
15	4.82	4.75	− 0.07	+ 0.23	+ 0.16
16	4.85	4.15	− 0.70	+ 0.26	− 0.44
17	4.90	4.30	− 0.60	+ 0.30	− 0.34
18	4.80	4.15	− 0.65	+ 0.19	− 0.46
19	4.85	4.18	− 0.67	+ 0.23	− 0.46
20	5.00	4.30	− 0.70	+ 0.24	− 0.46
21	5.00	4.43	− 0.57	+ 0.21	− 0.45
22	5.22	4.50	− 0.72	+ 0.26	− 0.44
23	5.27	4.65	− 0.62	+ 0.24	− 0.39
24	5.32	4.75	− 0.57	+ 0.22	− 0.31
Av.	4.599	4.061	− 0.538	+ 0.255*	− 0.290*

* Highly significantly different from zero.

other (table 6). The tests on the homogenized milk became consistently higher than those of the Mojonnier, averaging + 0.238, whereas the regular Pennsylvania tests were under those of the Mojonnier in 75 per cent of the cases. The wide variation encountered between the tests of nonhomogenized and homogenized milk when made by the Pennsylvania method were narrowed appreciably when sulfuric acid of 1.81 specific gravity was used in the test (table 6 and fig. 1). Nevertheless, the average readings were considerably above those of the Mojonnier (fig. 1).

The data for all tests are summarized in table 7 and figure 1.

TABLE 6
Comparison of modified Pennsylvania and Mojonnier tests on homogenized milk

Series	Modified Pennsylvania method			Variations from Mojonnier	
	Nonhomogenized	Homogenized	Variation of homogenized from non-homogenized.	Nonhomogenized	Homogenized
	%	%			
1	3.90	3.88	−0.02	+0.25	+0.21
2	3.95	3.90	−0.05	+0.25	+0.20
3	4.05	4.00	−0.05	+0.34	+0.30
4	4.08	4.08	0.00	+0.36	+0.35
5	4.15	4.13	−0.02	+0.34	+0.31
6	4.02	4.00	−0.02	+0.20	+0.19
7	4.15	4.15	0.00	+0.29	+0.32
8	4.22	4.20	−0.02	+0.32	+0.31
9	4.20	4.15	−0.05	+0.31	+0.24
10	4.63	4.55	−0.08	+0.24	+0.14
11	4.78	4.68	−0.10	+0.31	+0.18
12	4.77	4.75	−0.02	+0.24	+0.20
13	4.78	4.80	÷0.02	+0.20	+0.23
14	4.85	4.87	+0.02	+0.28	+0.28
15	4.87	4.85	−0.02	+0.28	+0.26
16	4.83	4.83	0.00	+0.24	+0.24
17	4.90	4.80	−0.10	+0.30	+0.16
18	4.87	4.82	−0.05	+0.26	+0.21
19	4.90	4.88	−0.02	+0.28	+0.24
20	5.03	4.93	−0.10	+0.27	+0.17
21	5.03	5.03	0.00	+0.24	+0.25
22	5.23	5.20	−0.03	+0.27	+0.26
23	5.30	5.23	−0.07	+0.27	+0.19
24	5.35	5.35	0.00	+0.25	+0.29
Av.	4.618	4.585	−0.032	+0.274*	+0.238*

* Highly significantly different from zero.

TABLE 7
Comparison of various fat tests of nonhomogenized and homogenized milk (Av. 24 trials)

Test	Percentage fat in		Variation of test of homogenized milk from that of nonhomogenized milk	Variation of test of homogenized milk from Mojonnier test
	Nonhomogenized milk	Homogenized milk		
Mojonnier	4.344	4.347	+0.003	....
Babcock*	4.402	4.390	−0.012	+0.043†
Gerber	4.434	4.437	+0.003	+0.090†
Minnesota	3.886	3.913	+0.027	−0.433†
Pennsylvania	4.599	4.061	−0.538	−0.290†
Pennsylvania‡	4.618	4.585	−0.032	+0.238†

* Acid and milk at 70° F., 17.5 ml. of 1.835 sp. gr. acid added in 3 portions, shaken by hand after each addition and finally shaken in shaking machine for at least 2 minutes.
† Highly significantly different from zero.
‡ Specific gravity of acid adjusted to 1.81 instead of the recommended specific gravity of 1.73.
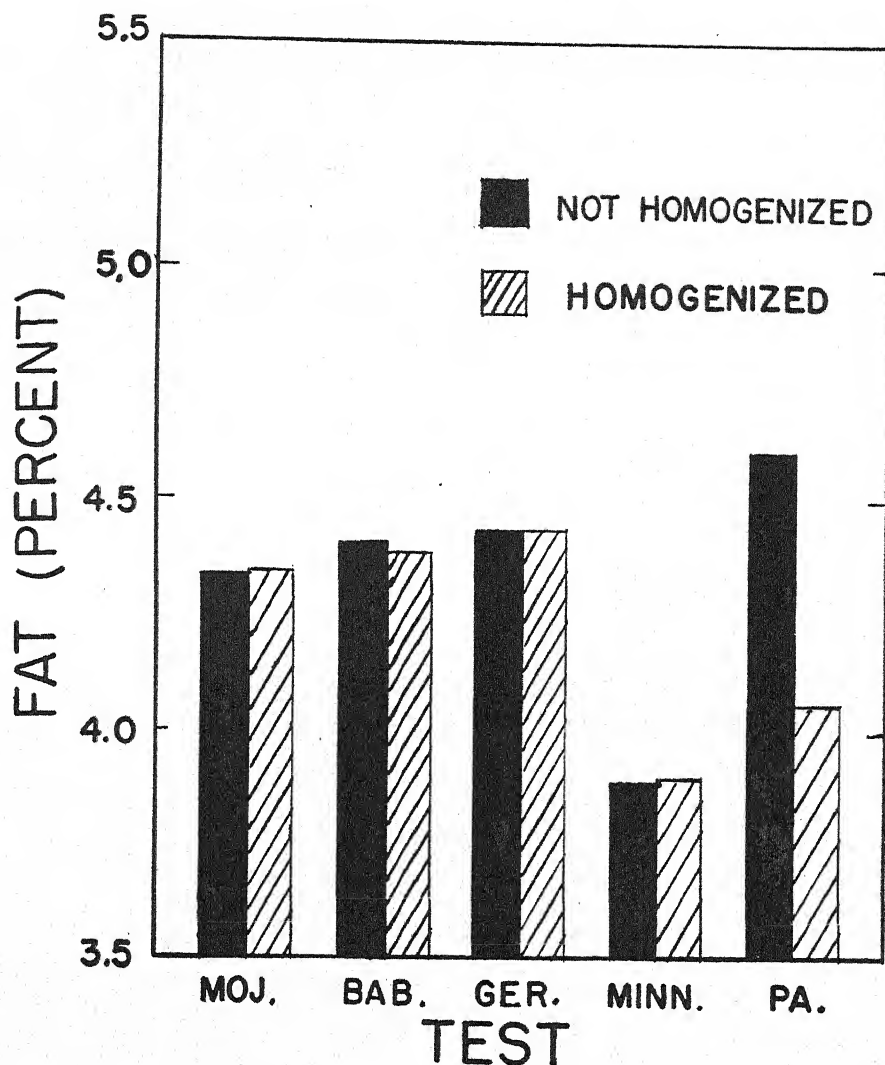
FIG. 1. Fat percentage in milk as determined by the Mojonnier, modified Babcock, Gerber, Minnesota, and Pennsylvania method (average 24 trials).

CONCLUSIONS

Homogenization does not affect the Mojonnier fat test of milk.

The modified Babcock method (17.5 ml. of 1.835 sp. gr. sulfuric acid added in three portions, 8, 5, and 4.5 ml., respectively, and shaken for at least 2 minutes before centrifuging) may be used with much assurance of accuracy in testing homogenized milk. Twenty-four tests in duplicate averaged within -0.012 per cent of those of nonhomogenized milk made by the same method and within $+0.043$ per cent of the Mojonnier average.

Homogenization does not affect the Gerber test. The average Gerber tests, both of nonhomogenized and homogenized milk, were found to be 0.09 per cent higher than those secured by the Mojonnier method. Aside from the necessity of introducing another test and the fact that the readings were approximately 0.1 per cent higher than the Mojonnier, the Gerber test was by all odds the most satisfactory test studied for making fat tests of homogenized milk.

While the Minnesota method yielded average tests of homogenized milk within + 0.027 per cent of those of nonhomogenized milk, the tests varied from those of the Mojonnier on the average by − 0.433 per cent. It would seem, therefore, that the test could not be recommended for testing homogenized milk.

The Pennsylvania method, yielding tests on homogenized milk in these studies markedly lower than the Mojonnier method, cannot be recommended for testing homogenized milk.

Credit is due Mr. Robert Frantz for the making of the tests and Dr. W. D. Baten for the testing of significance of some of the data reported herein.

REFERENCES

(1) Arizona Agricultural Experiment Station. The Babcock vs. the Mojonnier Test for Fat in Homogenized Milk. 52nd Ann. Rpt. Ariz. Agr. Expt. Sta., p. 54. 1941.

(2) Arizona Agricultural Experiment Station. Babcock vs. Mojonnier Test for Fat in Homogenized Milk. 55th Ann. Rpt. Ariz. Agr. Expt. Sta., pp. 49–50. 1944.

(3) BABCOCK, S. M. A New Method for the Estimation of Fat in Milk, Especially Adapted to Creameries and Cheese Factories. Wis. Agr. Expt. Sta. Bul. 24. 1890.

(4) BIRD, E. W., AND BREAZEALE, D. F. A Study of the Several Minnesota Reagents for the Determination of Fat in Buttermilk. Jour. Dairy Sci., 20: 1–7. 1937.

(5) VAN DER BURG, B. Accuracy of Fat Estimation by Gerber's Method (Trans. title). Off. Org. Alg. Ned. Zuivelbond. 1929. (Abst. Jour. Dairy Res., 1: 207. 1930.)

(6) BURR, A. Fettbestimmung in homogenisierter Milch. Milchw. Zentbl., 1: 6–9. 1905.

(7) BURR, A., AND WEISE, H. Untersuchung homogenisierter Milchflussigkeiten. Molk. Ztg. (Hildesheim), 28: 367–368, 381–382. 1914.

(8) BUTTENBERG, P. Über homogenisierte Milch. Ztschr. f. Untersuch. der Nahr. u. Genussmtl., 6: 964–968. 1903.

(9) DAHLBERG, A. C. Comparison of the Roese-Gottlieb and Babcock Methods of Testing. Assoc. Off. Agr. Chem. Jour., 7: 159–169. 1923.

(10) DAHLBERG, A. C. The Comparative Accuracy of the Gerber and Babcock Tests (Sub-committee rpt.), Jour. Dairy Sci., 10: 180. 1927.

(11) DAHLBERG, A. C., HOLM, G. E., AND TROY, H. C. A Comparison of the Babcock, Gerber and Roese-Gottlieb Methods for Determining the Percentage of Fat in Milk and Cream. N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 122. 1926.

(12) DOAN, F. J. Laboratory Control of Homogenized Milk. Internatl. Assoc. Milk Dealers Bulletin, Lab. Sect., pp. 315–343. 1943.

(13) FAHL, J. R., LUCAS, P. S., AND BATEN, W. D. Factors Involved in Accuracy of Testing Milk Samples. Mich. Agr. Expt. Sta. Tech. Bul. 158. 1938.

(14) FISHER, R. C., AND WALTS, C. C. A Comparative Study of Methods for Determining the Per Cent of Fat in Dairy Products. Conn. (Storrs) Agr. Expt. Sta. Bul. 131. 1925.

(15) HERREID, E. O. The Babcock Test: A Review of the Literature. Jour. Dairy Sci., 25: 335–370. 1942.

(16) HERREID, E. O., WHITMAN, D. W., AND SLACK, R. O. Accounting for Milk Fat. Vt. Agr. Expt. Sta. Bul. 512. 1944.

(17) HERRINGTON, B. L. Amyl Alcohol as a Source of Error in the Gerber Test. Jour. Dairy Sci., 16: 557–558. 1933.

(18) HILEMAN, J. L., RUSH, K. K., AND MOSS, C. The Relation of Errors in the Babcock Test to Losses in Cream Plants. Jour. Dairy Sci., 25: 373–379. 1942.

(19) HOYBERG. Eine Schnelle zur Bestimmung des Fettgehaltes homogenisierter Milch. Ztschr. f. Fleisch u. Milchyg., 19: 352–355. 1909.

(20) ISTAZ, C., AND VAN SOEST, G. L'homogenization du lait. Rev. Gén. du Lait., 6: 241–248. 1907.

(21) LAMPERT, L. M., AND BRANDON, J. H. The Babcock Fat Test for Homogenized Milk. Jour. Milk Technol., 8: 140–144. 1945.

(22) LUCAS, P. S., AND TROUT, G. M. A Suggested Modified Babcock Procedure for Testing Homogenized Milk. Jour. Dairy Sci., 30: 95–102. 1947.

(23) MARQUARDT, J. C. A Method for Determining Fat in Homogenized Milk. Milk Dealer, 31, 5: 24. 1942.

(24) MOJONNIER, T., AND TROY, H. C. The Technical Control of Dairy Products. Mojonnier Bros., Chicago, Ill., pp. 51–52. 1925.

(25) MOJONNIER, T. Testing Homogenized Milk for Butter Fat. Food, 21, 1: 9–10. 1945.

(26) PETERSEN, W. E. Dairy Science. J. B. Lippincott Co., New York, p. 542. 1939.

(27) PHILLIPS, C. A. A Comparison of the Babcock and Roese-Gottlieb (Mojonnier) Methods for Determination of Butter Fat in Milk. Jour. Dairy Sci., 6: 549–555. 1923.

(28) RICHMOND, H. D. The Estimation of Fat in Homogenized Milk. Analyst, 31: 218–219. 1906.

(29) SIEGFELD, M. Die Fettbestimmung en mechanisch bearbeiteter Milch. Molk. Ztg. (Hildesheim), 18: 39–40. 1904. (Original not seen. Abst. Rev. Gén. du Lait., 4: 85–86. 1904.)

(30) VON SOBBE, O. Die Bestimmung des Homogenizationsgrades der Milch. Milchw. Zentbl., 43: 503–506. 1914.

(31) SWOPE, W. D. Pennsylvania Method for Determining the Percentage of Fat in Dairy Products. Pa. Agr. Expt. Sta. Bul. 412. 1941.

(32) TROUT, G. M. Physical and Chemical Effects of Homogenization of Milk. B. W. Hammer Panegyric. Collegiate Press, Ames, Ia., pp. 140–153. 1937.

(33) TROUT, G. M., HALLORAN, C. P., AND GOULD, I. The Effect of Homogenization on Some of the Physical and Chemical Properties of Milk. Mich. Agr. Expt. Sta. Tech. Bul. 145. 1935.

(34) TROUT, G. M., AND LUCAS, P. S. A Comparison of Various Modifications of the Babcock Test for the Testing of Homogenized Milk. Jour. Dairy Sci., 28: 901–919. 1945.

(35) WEBSTER, H. C. Investigation and Study of Factors Involved in the Accuracy of Sampling, Preserving and Testing Milk for Butter Fat. Tech. Bul. Ontario Concentrated Milk Prod. Assoc., Toronto, p. 64. 1945.

(36) WILSTER, G. H., AND ROBICHAUX, R. P. Sampling, Preserving, Testing Milk. Oreg. Agr. Expt. Sta. Sta. Bul. 383. 1940.
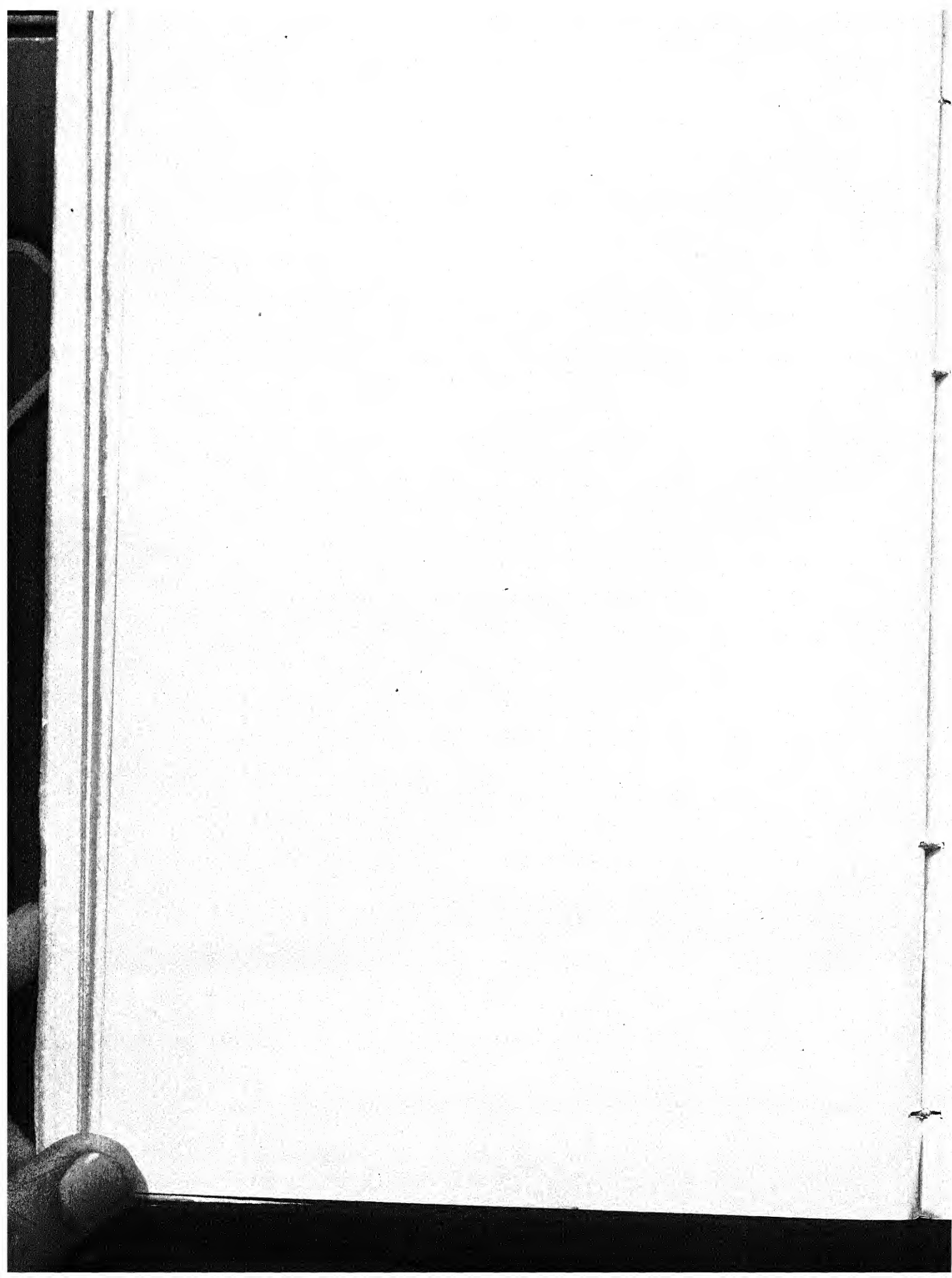
A METHOD FOR MEASURING THE BODY OF CULTURED CREAM

LUTHER D. HILKER

National Dairy Products Corporation Research Laboratories, Baltimore, Maryland

The body or viscosity of cultured cream has been measured by various investigators. After uniform agitation of the cultured cream, Guthrie (2) used a MacMichael viscosimeter to determine differences in the body of the product. Two methods were employed by Doan and Dahle (1), namely: (a) a viscosimeter of the rotating type and (b) "a special plunger test where the penetration of a suitable plunger falling a unit distance into the product was measured". No details were given as to the plunger or method of testing. Joffe (3) describes a method of testing the consistency of mayonnaise and salad dressing, two products which have a consistency similar to cultured cream. He used an instrument called a "plumit". This is a graduated rod of aluminum, bearing a pointed and weighted head of larger diameter than the rod. The total weight is 14.5 g. and the over-all length 13 cm. The plumit is held between the thumb and forefinger and dropped into a jar of mayonnaise from a height of 12 inches. When the plumit is released and drops into the sample, it should remain perpendicular, and a reading of the depth of penetration is taken at once.

Body structure or the apparent viscosity of cultured cream, developed without the addition of stabilizing agents, may be reduced readily to a basic viscosity by means of gentle agitation. It seems desirable to evaluate the uniformity of the product as sold to the consumer by measuring the body of the cultured cream while in the final container and without reducing the apparent viscosity. An aluminum rod, drilled, tapered, and graduated, has been constructed for this purpose. The size of the hole $(13/32'' \times 3\,3/4'')$ was selected to provide a desirable ratio of weight to volume displacement. The dimensions and mode of construction of this device, called a plummet, are given in figure 1. A release for the plummet (made from a glass tube and a board), a stop watch with a second hand, and a ring stand and clamp also are needed.

The procedure for conducting the test is as follows:

(a) Hold samples to be tested overnight at 40° F. If the retail container is less than 4 inches high and the exposed surface less than 2 inches in diameter, suitable containers (such as an 8-oz. mayonnaise jar) should be employed and filled and handled in a manner identical to the commercial product. Avoid agitating the product prior to testing.

(b) Firmly secure the glass tube in a vertical position with a suitable

clamp, having the end of the tube exactly 12 inches above the center surface of the cultured cream.

(c) Insert the plummet, point downward, in the glass tube and hold in
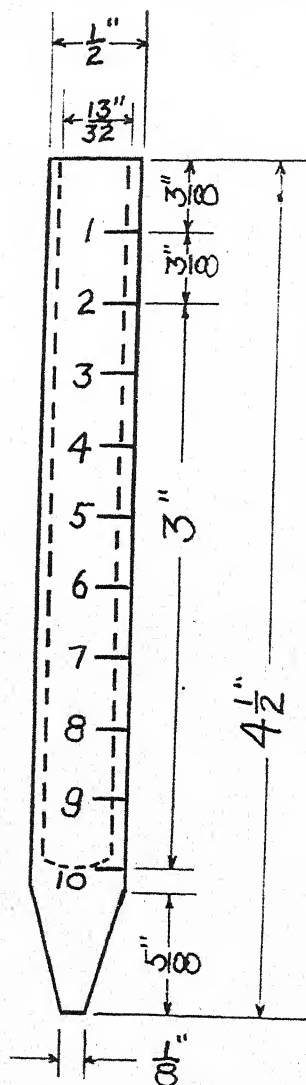
FIG. 1. Construction of plummet.

place with the smooth surface of the small board, maintaining a horizontal position against the bottom of the tube.

(d) Release the plummet by sliding the board quickly to one side.

(e) Lift the plummet from the cultured cream 5 seconds after its release.

(f) The cream will adhere to the plummet to a distance depending on the depth of penetration. Observe the cream line reading to the nearest quarter division of the scale. The cream line mark may not correspond to the true depth of penetration, due to the meniscus.

(g) An average of three tests is used as the measure of the body of the product. Use a different container of product for each test.

(h) The plummet should be washed and dried after each test.

TABLE 1

The body of cultured cream as measured by the plummet method

Plant no.	Batch no.	Plummet reading				Maximum deviation between individual sample containers
		Container no.			Av.	
		1	2	3		
1	1	7.50	7.25	7.25	7.33	0.25
	2	7.75	7.75	7.75	7.75	0.00
	3	7.25	7.50	7.50	7.42	0.25
	4	8.00	8.25	8.25	8.17	0.25
	5	7.75	8.25	8.00	8.00	0.50
	6	7.50	7.50	7.50	7.50	0.00
2	1	5.75	6.00	6.00	5.92	0.25
	2	7.00	7.25	7.00	7.08	0.25
	3	6.25	6.75	6.00	6.33	0.50
	4	7.00	7.25	6.50	6.91	0.75
	5	7.50	7.00	6.50	7.00	1.00
	6	7.25	6.75	7.00	7.00	0.50
3	1	5.00	5.50	5.25	5.25	0.50
	2	5.50	5.25	5.75	5.50	0.50
	3	6.75	7.25	6.50	6.83	0.75
	4	4.50	4.25	3.75	4.16	0.75
	5	4.75	4.25	4.50	4.50	0.50
	6	5.00	4.50	4.75	4.75	0.50
4	1	6.50	6.50	7.00	6.66	0.50
	2	8.00	8.50	8.00	8.16	0.50
	3	5.50	6.25	5.75	5.83	0.75
	4	7.75	8.00	....	7.87	0.25
	5	< 0	< 0	< 0	....	....
	6	7.00	7.50	7.00	7.16	0.50
5	1	4.50	4.25	4.00	4.25	0.50
	2	6.00	5.75	6.50	6.08	0.75
	3	5.75	5.50	5.75	5.66	0.25
	4	5.00	4.50	4.50	4.66	0.50
	5	6.00	5.50	5.50	5.66	0.50
	6	6.00	6.00	5.50	5.83	0.50
6	1	3.25	3.50	3.25	3.41	0.25
	2	3.75	3.50	3.50	3.56	0.25
	3	4.00	4.25	3.75	4.00	0.50
	4	3.50	3.50	3.75	3.56	0.25
	5	3.50	3.50	3.50	3.50	0.00
	6	3.25	3.50	3.50	3.41	0.25

Data presented in table 1 give the body of cultured cream as produced by six dairy plants located in eastern United States. Examination of these data indicates that the variations existing between measurements of indi-

vidual containers of the same lot of cultured cream are within the practical limits of controlled testing. A variation of ± 0.5 division is considered a suitable tolerance range. Daily tests on the body of cultured cream may be observed to indicate any variations from a predetermined standard.

The consensus of three experienced judges in correlating the visual viscosity of cultured cream with the plummet reading may be found in table 2.

TABLE 2

Relation of plummet readings to visual viscosity of cultured cream

Plummet reading	Visual viscosity
0 – 2	Very thin
2 – 4	Thin
4 – 6	Medium
6 – 7.5	Good
7.5– 8.5	Slightly heavy
8.5–10	Heavy
Over 10	Very heavy

SUMMARY

A simple method has been developed for measuring the body of cultured cream. The resistance of this product to the penetration of a plummet is taken as an indication of the body.

REFERENCES

(1) DOAN, F. J., AND DAHLE, C. D. The Manufacturing Process for Commercial Sour Cream. Pa. Agr. Expt. Sta. Bul. 233. 1928.

(2) GUTHRIE, E. S. Body of Cultured Cream. N. Y. (Cornell) Agr. Expt. Sta. Bul. 666. 1937.

(3) JOFFE, M. H. Mayonnaise and Salad Dressing Products. The Emulsol Corporation, Chicago. 1942.

THE FLAVOR, VOLATILE ACIDITY, AND SOLUBLE PROTEIN OF CHEDDAR AND OTHER CHEESE[1]

A. C. DAHLBERG AND F. V. KOSIKOWSKY

Department of Dairy Industry, Cornell University, Ithaca, N. Y.

The method of manufacturing Cheddar cheese has been known for many generations. About 50 years ago scientists began an intensive study of the factors affecting the flavor of this cheese with the idea that the source and identity of the flavoring substances could be established. Although much scientific knowledge has been accumulated from these studies, the specific flavoring materials are still unknown. The present trend toward the manufacture of Cheddar cheese from pasteurized milk has accentuated the need for this knowledge, as pasteurized milk cheese has less flavor and slightly different characteristics than that found in raw milk cheese.

The literature is voluminous and only a few references will be cited. The fundamental chemical changes that occur during the ripening process as known in 1891 were stated by Van Slyke (17). He wrote that there was a slow evolution of carbon dioxide from the casein or fat, or both. Volatile and nonvolatile fatty acids developed from the fat. The nitrogen compounds, especially casein, broke down into soluble compounds, some eventually becoming ammonia. Cured cheese was more alkaline than fresh cheese. The formation of the free fatty acids was the principal chemical change during ripening.

Van Slyke, Harding, and Hart (18) concluded from their study of rennet that this enzyme did not decompose protein into compounds that produced flavor in cheese. Suzuki, Hastings, and Hart (16) studied the origin and composition of steam distillate from cheese which contained the flavor compounds. They found bacteria to be the principal ripening agent. The lactose disappeared from cheese in 3 to 6 days but some of the lactates were fermented into volatile fatty acids, especially acetic and propionic. Butyric and caproic acids were derived from the fats and proteins. Succinic acid, alcohols, and esters also were present in the steam distillate.

Improved Cheddar cheese flavor has been reported with special cultures in Cheddar cheese making in addition to the usual lactic starter. Hucker and Marquardt (7) found that a *Streptococcus paracitrovorus* culture used with Hansen's commercial starter produced characteristic cheese flavor of superior quality from pasteurized milk. Proteolytic coccus cultures produced bitter flavor, as did the culture of *S. paracitrovorus* when used without starter. Hansen, Bendixen, and Theophilus (3) confirmed that cheese

made with *S. paracitrovorus* alone became bitter, and they obtained similar results with *Streptococcus citrovorus*. However, the quality of cheese made from raw or pasteurized milk with lactic starters was not improved by the addition of *S. paracitrovorus* or *S. citrovorus*. Definite improvement in the quantity and quality of Cheddar cheese flavor was obtained by Lane and Hammer (8) when cultures of selected strains of *Lactobacillus casei* were used in pasteurized milk to which commercial lactic starter had been added. The protein decomposition was greater and the flavor improved due to this bacterium. More recently, Sherwood (13, 14) isolated 720 strains of lactic acid bacteria from pasteurized milk Cheddar cheese in New Zealand. The dominant organism was *Lactobacillus (Streptobacterium) plantarum*, with *Lactobacillus (Streptobacterium) casei* occurring less frequently. Betabacteria and betacocci were found in small numbers. Some strains of these bacteria produced good Cheddar cheese flavors. Studies in the laboratory and in factories verified Lane and Hammer's good results (8) with a special strain of *L. casei*, but best cheese flavor was obtained by the addition of 10 ml. of culture of *L. plantarum* to 80 gallons of milk to give an inoculation of about 12,000 organisms per ml. of pasteurized milk.

The rôle of the hydrolysis of milk fat and the development of volatile acids in relation to flavor has been emphasized by the studies of Lane and Hammer (9, 10, 11). The homogenization of milk for the manufacture of blue cheese improved the texture and increased volatile acidity and flavor. In Cheddar cheese the volatile acidity and flavor were greater for raw milk cheese than for pasteurized milk cheese. Homogenization of the milk or the addition of lipase from several sources generally produced rancid, bitter cheese, but these flavors tended to disappear with age. Desiccated mammary tissue added to cheese milk often produced desirable Cheddar flavor. Babel and Hammer (1) found that the lipase in mulberry juice or rennet extract often did improve the flavor of Cheddar cheese, but too much of the enzyme produced bitter flavor. Dahle and Watrous (2) based their new procedure for making Parmesan-type cheese on the fact that homogenization of the milk promoted the development of the rancid flavor and aroma desired in this grating cheese for flavoring spaghetti and soups.

Cheese ripening generally is followed by increased volatile fatty acids and increased soluble nitrogen. Both chemical changes are used to indicate the degree of ripening as associated with the development of flavor, and increased soluble nitrogen with the development of a mellow, waxy body. The usual steam distillation of cheese for volatile acids does not give correct results, according to Hiscox, Harrison, and Wolf (4, 5, 6) who developed a long, accurate method for estimating these acids. The accurate, rapid technic of Smiley, Kosikowsky, and Dahlberg (15) has made it possible easily to analyze commercial cheese for total volatile acidity and to compare such values with flavor scores and intensities.

This study was undertaken to show the relationship of total volatile acidity to flavor, and consideration also was given to several other chemical determinations, including soluble nitrogen.

METHODS

Five cheese manufacturers or processors in New York and Wisconsin were requested to select three Cheddar cheeses that represented mild, medium, and sharp flavors of excellent quality. Each sample was to be selected as being a typical good Cheddar cheese. The cheese selectors were cautioned to avoid choosing the cheese on the basis of age and to depend solely upon examination of the cheese. In one case the three cheeses received from one manufacturer were made at one plant.

As the 15 samples were received, they were classified as mild, medium, or sharp in flavor. After all had been received, they were arranged in order of the intensity of the Cheddar flavor by the authors working independently and then together. The samples were analyzed for volatile acidity, soluble protein, pH, moisture, and salt.

Samples of cheese of several varieties other than Cheddar were purchased at a retail grocery store. These samples were classified as to flavor intensity and analyzed chemically. They were rated on the basis of intensity of flavor within their respective varieties and also without regard to variety.

Finally, a manufacturer of Camembert cheese was asked to select several samples that were approximately the same age and had been treated similarly but which showed differences in the degree of ripeness and intensity of flavor. The four samples were scored and classified by the manufacturer and his comments were confirmed by the authors.

The total volatile acidity was determined by the method of Smiley, Kosikowsky, and Dahlberg (15), and the pH by the Beckman pH meter, using glass electrodes.

The solution of the soluble protein followed a technic based upon the method of Sharp (12). The principle of the method is to maintain the pH (approximately 5) and the salt content comparable to that of Cheddar cheese by a buffer salt solution while the soluble protein is being dissolved in the water. The soluble protein was extracted as follows: Three grams of cheese are weighed within 0.01 g. error and placed in a porcelain mortar. A small amount of extracting solution at 50° C. is added and the cheese is ground to a thick paste. Additional solution is added to dilute the paste. The dilute suspension of cheese is transferred to a 100-ml. flask. The mortar is rinsed with additional portions of the solution. The flask is placed in a water bath at 50° C., filled to the mark with extracting solution, and, with occasional shaking, maintained at this temperature for one hour. The solution is filtered through a fluted filter, and 50 ml. of the filtrate is placed in a Kjeldahl flask.

The soluble protein extraction solution is prepared as follows:

A. Stock solution

> 57.5 ml. glacial acetic acid
> 136.1 g. sodium acetate (3 H_2O)
> 47.0 g. sodium chloride
> 8.9 g. calcium chloride (anhydrous)

Add water to make 1 liter.

B. Extraction solution

Make 250 ml. stock solution up to 1 liter with water.

RESULTS

Selected Cheddar Cheese

The data on Cheddar cheese, presented in table 1, do not show any striking relationships of intensity of cheese flavor with any of the factors studied. In a general way it may be stated that the comments of the manufacturers of the cheese agreed well with those of the authors as to the intensity of the cheese flavor. The scores of the cheese showed that all samples were good Cheddar cheese but the scores were not related to intensity of flavor.

It was true that the two oldest samples of cheese (E1 and A2 which were 13 and 14 months of age) were the highest in flavor intensity. However, age of cheese cannot be segregated from temperature of storage, for curing cheese is a time-temperature problem and this temperature was not reported. This probably explains the reason that the cheese (B3 and B4) which rated third and fourth in strength of flavor were among the youngest, 2 and 3 months old, for the buyer of this cheese often force-cures it at about 60° F., as compared with the usual storage temperature of 32 to 35° F. For this reason and others, flavor intensity and age of cheese were not associated closely enough to be able to state that a given cheese was strong in flavor because it was old (10 to 12 months) or that another was mild in flavor because it was young (2 to 4 months).

The principal object of this study was to establish the relationship between the intensity of Cheddar cheese flavor and total volatile acidity. There were two samples with volatile acidities below 20 ml. N/10 acid per 100 g. of cheese. These samples were 13 and 7 months old and were first and fourteenth in flavor intensity. There were three samples with volatile acidities over 40. These samples were 14, 2, and 2 months old and were second, seventh, and eleventh in intensity of flavor. With the two cheeses of lowest volatile acidity representing the most and next to the least flavor intensity, and the cheeses of highest volatile acidity also scattered as to flavor intensity, it is evident that a sample of Cheddar cheese could not be classed as to flavor intensity on the basis of total volatile acidity. There appears

TABLE 1

The relationship of flavor intensity, volatile acidity, soluble protein, and other constants in commercial Cheddar cheese of good flavor

Sample no.*	Age of cheese	Raw or past. milk	Comment on flavor		Authors' score	Order of flavor intensity	Volatile acidity†	Soluble protein	pH	Mois- ture	Salt
	(mos.)		Mfgr's.	Authors'				(%)		(%)	(%)
E1	13	Raw	Sharp	Sharp	94	1	16.8	8.40	5.10	35.56	1.33
A2	14	Past.	Sharp	Sharp	92	2	48.0	8.88	5.20	35.91	1.41
B3	3	Raw	Sharp	Sharp–	92	3	29.2	5.56	5.00	39.01	1.87
B4	3	Raw	Medium	Sharp–	91	4	32.6	5.53	5.06	37.73	1.45
C5	12	Raw	Medium	Medium+	90.5	5	18.8	8.17	5.33	33.60	2.00
D6	12	Past.	Sharp	Mild+	93	6	24.0	7.27	5.51	35.95	2.13
B7	2	Raw	Medium	Medium+	90	7	40.5	4.92	5.18	37.49	1.39
C8	9	Raw	Sharp	Medium	91.5	8	25.8	7.47	5.10	35.68	1.98
B9	3	Raw	Mild	Medium	91	9	23.2	7.12	5.30	39.99	1.58
D10	9	Past.	Medium	Medium–	92	10	24.0	5.81	5.28	37.25	1.48
A11	2	Raw	Mild	Mild+	91.5	11	40.8	4.82	5.00	34.26	1.23
A12	10	Past.	Medium	Mild+	92	12	28.3	7.15	5.08	33.97	1.03
D13	4	Past.	Mild	Mild+	92	13	25.0	4.53	5.39	37.79	2.05
E14	7	Past.	Mild	Mild	92	14	13.7	6.68	5.25	37.53	1.48
C15	3	Raw	Mild	Mild	92	15	21.7	5.56	5.49	35.64	1.48

* The letter refers to the cheese manufacturer or processor who selected the samples.

† Ml. N/10 acid per 100 g. cheese.

to be almost no general trend in the relationship between volatile acidity and flavor intensity.

The data on the relationship between soluble protein and flavor intensity did not happen to be so strikingly negative as was the case for total volatile acidity. Samples E1 and A2, with the greatest amount of soluble protein, 8.40 and 8.88 per cent, respectively, were oldest and ranked first and second in intensity of Cheddar cheese flavor. However, the relationship between the two factors appeared to end with these two samples. Samples with less than 5 per cent soluble protein were seventh, eleventh, and thirteenth in
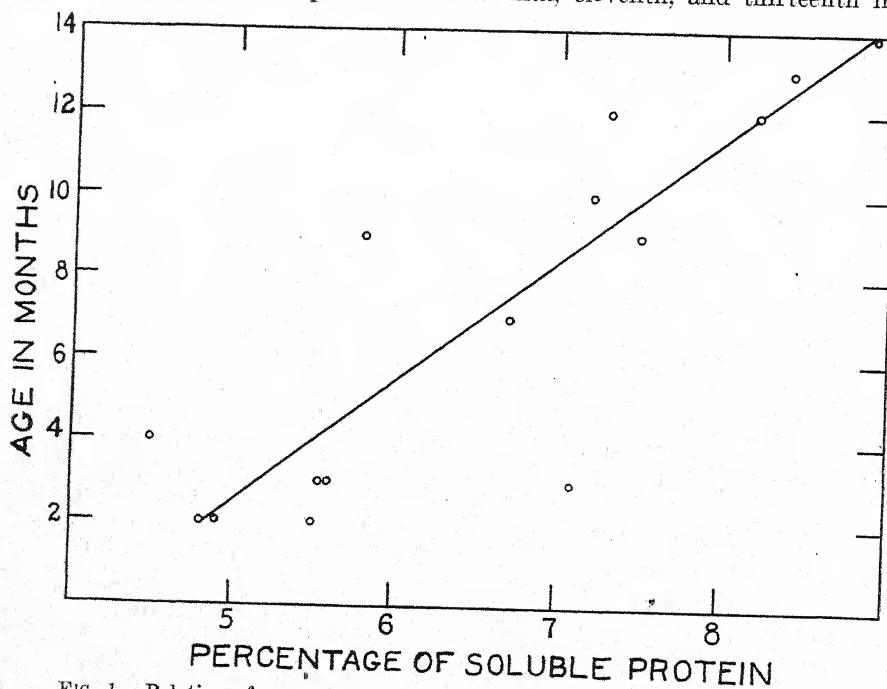
FIG. 1. Relation of percentage of soluble protein to age of Cheddar cheese.

flavor. Samples with 5 to 6 per cent soluble protein were third, fourth, tenth, and fifteenth in flavor intensity. There was no obvious trend in the relationship between these two factors.

The percentage of soluble protein increased with increased age of the cheese (fig. 1). This trend had some exceptions but it was reasonably good considering the varied character of the cheese. A similar relationship did not exist between total volatile acidity and age of the cheese.

There is nothing in the data to suggest any correlation of flavor intensity with the pH, moisture, and salt content of the cheese.

Miscellaneous Cheese Varieties

The miscellaneous varieties of cheese purchased at a grocery store gave

TABLE 2

*The relationship of volatile acidity, pH, and the quality and intensity
of flavor of various types of cheese*

Cheese variety	Cheese characteristics			Volatile acidity*	pH
	Relative intensity of flavor of all cheese	Flavor in each individual cheese class			
		Intensity of flavor	Quality remarks		
Camembert	1	Strong	Overripe	39.9	7.43
Blue	2	Medium	Good	40.4	6.00
Kaukauna Klub Cheese Food	3	Medium	Good	30.8	5.36
Liederkranz	4	Strong	Good	102.8	7.27
D'Oka (Trappist)	5	Strong	Fair	49.2	5.90
Chantelle	6	Medium	Fair	31.4	5.23
Swiss	7	Mild	Good	49.5	5.57

*Ml. N/10 acid per 100 g. cheese.

very interesting data (table 2). A high-flavored, overripe, soft Camembert cheese with an ammonia odor was rated as having the strongest flavor. It was alkaline in pH. A green, mild but good-flavored Swiss cheese was rated lowest in flavor; yet the total volatile acidity of the Swiss was greater than that of the Camembert. A blue cheese of medium-strength flavor of good quality was rated second in flavor intensity and its volatile acidity was the same as that of the Camembert. A strong-flavored Liederkranz cheese that was slightly alkaline contained more than twice as much volatile acidity as any other cheese; yet it was not the strongest-flavored cheese. Its flavor was very much the same as the D'Oka cheese with only half the volatile acidity.

It is appreciated that this attempt to rate cheese of different varieties on intensity of flavor is subject to criticism. The flavors are so different that intensities cannot be compared closely. After making allowances for such discrepancies, there can be no doubt concerning the lack of relationship between total volatile acidity and intensity of flavor.

TABLE 3

*Analysis of four domestic Camembert cheeses from different lots
made in the same plant*

Cheese	Age	Manufacturer's grade		Volatile acidity*	Soluble protein	pH	Moisture
		Score	Comments				
	(days)				(%)		(%)
1	48	20.0	Mild flavor Slight flat Poor breakdown	20.4	5.84	6.63	52.03
2	45	21.0	Mild flavor Fair breakdown	16.1	6.00	6.78	53.56
3	48	21.5	Fair flavor Fair breakdown	18.6	6.53	7.01	51.00
4	47	22.0	Good cheese	21.4	6.41	6.95	51.93

* Ml. N/10 acid per 100 g. cheese.

Selected Camembert Cheese

The ripening of Camembert cheese is sometimes subject to unexpected flavor variations which might be associated with total volatile acidity and soluble protein. It is recognized that the softening of the cheese is dependent upon protein solubility. A manufacturer of Camembert selected four cheeses of approximately the same age but with marked variation in the amount of good Camembert flavor (table 3). The manufacturer's score and comments on the cheese were used for comparisons with analysis.

The total volatile acidities of the cheese with the least flavor and with the most flavor were practically identical. The percentage of soluble protein and the pH value increased as the flavor increased in intensity and the cheese became softer in body. It is possbile that the relationship between intensity of flavor and changes in soluble nitrogen and pH were incidental to the obvious change in the body of the cheese, which must be related to these changes.

DISCUSSION

The tendency toward the manufacture of cheese from pasteurized milk has emphasized the lack of knowledge of those chemical entities which are responsible for the characteristic flavor of cheese. The literature of the last century gave the two major chemical changes that occur during ripening, namely, the increase in total volatile and nonvolatile acidity, and the increase in soluble protein. The emphasis was given to volatile acidity more than to the decomposition products of protein. Many investigators have endeavored to increase cheese flavor by the bacteria and enzymes which affect the fat and the casein.

This research has established that the magnitude of these chemical changes is not related to the intensity of the flavor of Cheddar cheese. The increase in total volatile acidity and soluble nitrogen during ripening is interesting and valuable information to obtain on cheese ripening, but flavor development is not related to these characteristics. This means that much of the research on the development of cheese flavor has been based upon a fundamental misconception. It may be that the cheese flavor is a result of the decomposition of milk fat or protein, but, if such is the case, the flavor compounds are developed without regard to the total changes in these two materials. In other words, if the flavor compounds of Cheddar cheese are volatile fatty acids, then the quantity of the flavor-producing acids is not related to total volatile acidity. It has been assumed fallaciously that these two were synonymous, as the volatile acids contain most of the cheese flavor.

Cheese flavoring compounds are developed during ripening incidental to the common chemical changes with which investigators often are concerned. This means there is need for a widening of the concept behind the studies of cheese flavor. More rapid progress may be made by new manu-

facturing technics or by identification of the flavor compounds. There is real hope for producing higher flavors in pasteurized milk cheese. For example, sample A2 was a pasteurized milk Cheddar cheese that possessed second highest flavor and the highest volatile acidity and soluble protein. This cheese may have been an exception for the plant that made it, but the fact is that it was produced.

As cheese ripens there is an increase in flavor, volatile acidity, and soluble protein. The lack of any correlation among these progressive changes in Cheddar cheese, except for a general relationship between age and soluble protein, does not disprove their occurrence. Instead, it emphasizes that there are a number of factors which affect each of these changes in varying degrees and partially obscure the effect of age alone. The direct relationship between age and soluble protein might be explained as being due chiefly to the action of rennet as affected by time, with other factors being less important in their total effects. The effect of microorganisms on the development of flavor is of major importance and is influenced greatly by a number of factors other than variations in time.

CONCLUSIONS

Cheese manufacturers and processors in New York and Wisconsin selected 15 Cheddar cheeses with typical good flavors that were mild, medium, and sharp in intensity. The cheese varied from 2 to 14 months of age. There were no relationships among intensity of flavor, total volatile acidity, soluble protein content, pH, and age of the cheese, except for a direct relationship between age and soluble protein. The flavor compounds were distilled with the volatile acids but apparently were not directly correlated as to total amounts.

No relationship was found between intensity of flavor and total volatile acidity in seven different varieties of cheese secured at a retail store.

Four samples of Camembert cheese made in one factory were selected to show difference in flavor and texture. There was no relationship between intensity of flavor and total volatile acidity but there was a direct relationship between flavor intensity of this cheese and its soluble protein and pH values.

REFERENCES

(1) BABEL, F. J., AND HAMMER, B. W. Fat Degradation in Cheddar Cheese Made from Pasteurized Milk, without and with Added Lipase. Jour. Dairy Sci., 28: 201–208. 1945.

(2) DAHLE, C. D., AND WATROUS, G. H., JR. A Grating Type Cheese. Natl. Butter and Cheese Jour., 36: 44. Nov., 1945.

(3) HANSEN, H. C., BENDIXEN, H. A., AND THEOPHILUS, D. R. Influence of Different Starters on the Quality of Cheddar Cheese. Jour. Dairy Sci., 16: 121–127. 1933.

(4) HISCOX, E. R., AND HARRISON, J. Volatile Acids of Cheese. I. Retentive Power of Cheese and Its Constituents. Jour. Dairy Res., 9: 215–226. 1938.

(5) HISCOX, E. R., HARRISON, J., AND WOLF, J. Z.   Volatile Acids of Cheese.   II.
        Methods of Extraction. Jour. Dairy Res., 9: 227–232. 1938.

(6) HISCOX, E. R., HARRISON, J., AND WOLF, J. Z.   Volatile Acids of Cheese.   III.
        Application of the Extraction Method. Jour. Dairy Res., 12: 155–169. 1941.

(7) HUCKER, G. J., AND MARQUARDT, J. C. The Effect of Certain Lactic Producing
        Streptococci upon the Flavor of Cheddar Cheese. N. Y. (Geneva) Agr. Expt.
        Sta. Tech. Bul. 117. 1926.

(8) LANE, C. B., AND HAMMER, B. W.   Bacteriology of Cheese.   II. Effect of *Lacto-
        bacillus casei* on the Nitrogenous Decomposition and Flavor Development in
        Cheddar Cheese Made from Pasteurized Milk. Iowa Agr. Expt. Sta. Res. Bul.
        190. 1935.

(9) LANE, C. B., AND HAMMER, B. W.   The Manufacture of Blue Cheese (Roquefort
        Type) from Homogenized Cows' Milk. Iowa State Col. Jour. Sci., 10: 391–393.
        1936.

(10) LANE, C. B., AND HAMMER, B. W.   Studies on the Fat of Cheddar Cheese. Iowa
        State Col. Jour. Sci., 13: 149–155. 1939.

(11) LANE, C. B., AND HAMMER, B. W.   Bacteriology of Cheese.   VI. Relationship of
        Fat Hydrolysis to the Ripening of Cheddar Cheese. Iowa Agr. Expt. Sta. Res.
        Bul. 291. 1941.

(12) SHARP, P. F.   Private Correspondence, about 1940.

(13) SHERWOOD, I. R.   The Bacterial Flora of New Zealand Cheddar Cheese. Jour.
        Dairy Res., 10: 426–448. 1939.

(14) SHERWOOD, I. R.   Lactic Acid Bacteria in Relation to Cheese Flavour.   II. Obser-
        vations on the Inoculation of the Milk Employed in Cheese Manufacture with
        Lactobacilli. Jour. Dairy Res., 10: 449–454. 1939.

(15) SMILEY, K. L., KOSIKOWSKY, F. V., AND DAHLBERG, A. C.   A Simplified Extraction-
        Distillation Method for the Determination of the Volatile Fatty Acids of Cheese.
        Jour. Dairy Sci., 29: 307–315. 1946.

(16) SUZUKI, S. K., HASTINGS, E. G., AND HART, E. B.   The Production of Volatile
        Fatty Acids and Esters in Cheddar Cheese and Their Relation to the Develop-
        ment of Flavor. Wis. Agr. Expt. Sta. Res. Bul. 11. 1910.

(17) VAN SLYKE, L. L.   Investigation of Cheese. N. Y. (Geneva) Agr. Expt. Sta. Bul.
        37. 1891.

(18) VAN SLYKE, L. L., HARDING, H. A., AND HART, E. B.   Rennet-Enzyme as a Factor
        in Cheese-Ripening. N. Y. (Geneva) Agr. Expt. Sta. Bul. 233. 1903.

THE VALUE OF SUPPLEMENTARY VITAMIN FEEDING IN THE REARING OF DAIRY CALVES

W. B. NEVENS AND K. A. KENDALL

Illinois Agricultural Experiment Station, Urbana, Illinois

Widespread interest in the vitamin needs of dairy calves was aroused through several reports (5, 6, 9, 10) that supplementary vitamin feeding during the first few weeks of life prevented so-called "nutritional scours", lessened navel infection, and reduced the death rate. The studies pointed to vitamins A and C and nicotinic acid as essential components of the ration of the young calf. The reported beneficial effects of vitamin supplementation were so striking that it was considered desirable to test the value of such a procedure in a dairy herd in which good feeding practices are followed throughout the year. On the basis of a number of published studies showing a relation between the character of the ration and the vitamin content of colostrum milk, it was assumed that the mother's ration during the gestation period also may affect the store of vitamins in the body of the calf at birth. Recent reports (3, 11, 14) tend to substantiate the validity of such an assumption.

A further assumption based on numerous calf-raising trials is that the most critical period in the life of the hand-fed dairy calf is within the first 30 days after birth. An early investigation in the feeding of dairy calves conducted at this Station (1) indicated that the character of the ration during the first 3 weeks is of prime importance. The data reported in this paper, therefore, cover only records up to 30 days of age. Histories of 299 calves, approximately one half of them males, are summarized.

PROCEDURE

The study was carried out with calves born in the Station dairy herd. The cows in this herd were fed hay, silage, and grain mixture throughout the year, with limited access to pasture during the growing season. The quality of all feeds, as a rule, was much above average. Alfalfa hay was the chief dry roughage, with smaller amounts of red clover hay and Korean lespedeza hay. The calves usually were left with their mothers for 2 days after birth, but some weak calves were left as long as 4 days. After separation from their dams they were kept in special quarters at two different locations. Feeding of whole milk (Holstein) was continued throughout the 30-day period of observation covered by these records. The calves were given free access to adequate amounts of red clover hay and a grain mixture. Live-weights were taken as early as possible after birth. However, some calves were born when no attendant was present, and probably a part of these had opportunity to nurse before they were weighed. These weights, therefore,

are termed first-day weights, rather than birth weights. Liveweights also were taken at 15 days and at 30 days of age.

The vitamin supplements were supplied in two forms. Nopco special A and D feeding oil with a potency of 2,000 units of A and 800 units of D per gram was supplied at the rate of approximately 2 ml. per head daily to all calves at one barn for the first half of the experimental period and none was given during the second half of the period. At the other barn the practice was reversed. Approximately equal numbers of calves were included in the groups fed oil and no oil. The other form of supplement consisted of vitamin capsules, the formulae for which were as follows:

	Black capsule	White capsule
Vitamin A, U.S.P. units	5,000	5,000
Vitamin D (Irradiated ergosterol), U.S.P. units	500	500
Nicotinic acid, mg.	50	50
Vitamin C (Ascorbic acid), mg.	250	....

Random selection of the calves to be fed the vitamin capsules was achieved by following the rule that the first calf born was to be fed vitamin capsules, the next one to be given no vitamin capsules, and so on. Thus one half of the calves were fed vitamin capsules and the remainder served as controls. One capsule containing vitamins A and D, nicotinic acid, and ascorbic acid was given daily per calf from 1 to 10 days of age and one capsule containing only vitamins A, D, and nicotinic acid was administered daily from 11 to 30 days of age.

Four experimental groups were established. Group I received no vitamin supplement, group II received the vitamin capsules, group III received the vitamin A and D oil supplement and group IV received both the vitamin capsules and the oil supplement.

An individual record sheet for each calf was posted on a bulletin board and records kept of the vitamins and milk fed, liveweights, condition of the calf, and, in the event that scours occurred, the treatment given and the duration of the ailment. The trials reported in this paper began early in 1944 and extended through September, 1946.

RESULTS

A summary of the records of all calves which survived the 30-day observation period is given in table 1. Characteristic breed differences were noted in the first-day weights of the calves. The average weights were: Ayrshires, 76 lbs.; Brown Swiss, 98 lbs.; Guernseys, 66 lbs.; Holsteins, 96 lbs.; and Jerseys, 50 lbs. The average gains in liveweight were somewhat greater for the Brown Swiss and Holsteins than for the other breeds. All of the average gains, however, were satisfactory for the breeds represented with the exception of those for the Guernseys in group II. This group included three calves which made very small gains. The average 30-day

TABLE 1

Effects of vitamin supplementation on liveweight gains and incidence of scours in dairy calves

Breed*	No. of calves	Av. values per head				Scours		
		First-day weight	Gain in weight		Milk fed	Number of cases	Av. age began	Av. duration
			To 15 days	To 30 days				
		(lbs.)	(lbs.)	(lbs.)	(lbs.)		(days)	(days)
I. Calves fed no vitamin supplement								
A	9	79.1	7.0	18.1	258	3	9	2
B.S.	5	104.9	9.4	20.3	233			
G	7	62.5	7.9	19.9	202	1	12	2
H	35	95.6	9.3	21.0	234	8	7	4
J	12	45.3	8.5	17.7	173	4	9	2
All	68	81.8	8.7±0.32	19.9±0.41	223	16	8	3
Coefficient of variability			43.9±0.36	24.8±0.21				
II. Calves fed vitamin capsules								
A	12	75.4	6.9	18.7	238	6	12	5
B.S.	10	97.8	10.1	21.0	214	2	6	3
G	14	62.6	4.5	12.9	183	3	9	3
H	32	97.7	8.6	20.8	234	7	8	3
J	8	49.3	8.2	18.1	184	1	9	3
All	76	82.6	7.7±0.42	18.8±0.97	217	19	9	4
Coefficient of variability			70.0±0.54	45.0±0.35				
III. Calves fed vitamin A and D oil concentrate								
A	7	75.7	9.9	23.6	244	1	19	3
B.S.	8	94.2	12.9	26.4	221	2	8	2
G	6	70.3	8.5	18.3	218	1	14	3
H	36	98.1	10.8	25.1	260	10	10	2
J	9	51.8	9.6	20.9	187	6	6	2
All	66	86.4	10.6±0.35	23.9±0.55	239	20	9	2
Coefficient of variability			39.6±0.33	27.3±0.23				
IV. Calves fed vitamin capsules plus vitamin A and D oil concentrate								
A	4	69.8	10.7	23.3	242	1	21	2
B.S.	8	97.4	11.1	25.6	245			
G	7	71.9	7.8	21.6	216	1	19	1
H	36	91.7	10.1	26.2	261	7	6	3
J	9	53.3	9.3	19.7	198	2	4	2
All	64	83.5	9.8±0.36	24.5±0.57	243	11	8	2
Coefficient of variability			42.8±0.37	27.2±0.24				

* A, Ayrshire; B.S., Brown Swiss; G, Guernsey; H, Holstein; J, Jersey.

gain for the 11 other Guernseys in this group was 16 lbs., a satisfactory figure.   The gains for the calves in groups III and IV were larger than those for groups I and II, but the amounts of milk fed to groups III and IV also were larger.

The four groups of calves were similar with respect to the number of cases of scours, age at which the attacks of scours occurred, and the duration of the ailment.

A summary of the records of calves which survived the 2-day nursing period and were started on hand feeding but which failed to complete the 30-day experimental period is given in table 2.   Records of calves born dead

TABLE 2

History and apparent cause of death of calves which failed to survive the 30-day observation period

	Group and kind of vitamin supplementation			
	I None	II Vitamin capsules	III A and D feeding oil	IV Capsules and A and D feeding oil
No. of calves	8	6	6	5
Average age at death, days	16	9	14	12
Death attributed to:				
Accidents	......	1	......	......
Indigestion and scours	1	1*	1	1
Pneumonia	1	......	2	2
Scours and pneumonia	3	1	......	......
Too weak to stand or nurse	1	1	1	2
Very weak at birth	2	2	1†	......
Weak at birth; died from bloat	......	......	1	......

* Mother had mastitis.
† Mother had preparturient mastitis.

and of those that died within 48 hours are not included.   Death was attributed to a number of causes, among which pneumonia alone and also scours accompanied by or followed by pneumonia were important.   There was little difference between the groups, however, in the number of calves affected.   Only two of the calves in group I died at less than 12 days of age. One calf in group I and one calf in group IV died of pneumonia on the 30th day.   It was noted that in a considerable number of cases scours did not occur until the calves had reached an age of 2 to 3 weeks.

DISCUSSION

Considerable variability in liveweight gains occurred among the calves of the four groups.   The gains to 15 days of age showed greater variability than the gains to 30 days.   This was an expected difference because more cases of scours occurred prior to 15 days of age than between 15 and 30 days and, also, some calves which were weak at birth and made a slow start made

good gains after 15 days.   The variability of the gains to 30 days of age was, surprisingly, somewhat less than that found at this Station for pasture-fed yearling dairy heifers (7).

The use of vitamin supplements was not effective in reducing the number of cases of scours, the duration of the ailment, and the mortality rate below those of the controls (group I).   It appeared that weakness at birth and inability to stand or nurse, the presence of infectious mastitis in the udder of the mother, accidents, and exposure to cold followed by pneumonia were important contributing causes of illness and death.

The use of capsules containing vitamins A and D, together with nicotinic acid and ascorbic acid, was no more effectual in promoting gains in weight or in reducing scours than the use of the special feeding oil containing only vitamins A and D, as judged by the records of calves included in groups II, III, and IV.   It is concluded, therefore, that the supplementation of the whole milk ration with ascorbic acid and nicotinic acid was ineffectual in promoting the well-being of the calves in this herd.   This finding is in agreement with recent investigations at this Station (4, 12) which showed that ascorbic acid and nicotinic acid are not required by the dairy calf.   On the other hand, the same technics used in these investigations disclosed the fact that riboflavin is required by dairy calves (13).   These several experiments lend no support to the inference given in a recent report (2) that the supplementary effect of nicotinic acid is dependent upon the level of vitamin A intake.

Our investigations lead us to the conviction that a measure of the progress of the calf during the first 30 days after birth is a better index of nutritional status in early life than is any other period.   The character of the ration after 30 days of age may be much more varied than prior to that time.   The consumption of high-quality hay and special grain mixture, both of which may be good sources of vitamins and other nutrients, tends to correct nutritional deficiencies incurred during the first few weeks of life.   In a recent report of controlled experiments with young calves (8), it is shown that 35 of 57 calves developed cases of scours, but only one of the cases that occurred after 4 weeks of age affected a calf not previously afflicted with the ailment.

Our results do not preclude the possibility that nutritional deficiencies may have been responsible for the onset of scours and other ailments in the case of some calves, particularly those which were too weak at birth to nurse and those which were not strong enough to obtain adequate amounts of colostrum milk.   Further, the desirability of vitamin A and D supplementation in the rations of calves born in herds where the rations are low in vitamin value is not disproved.   It is assumed on the basis of recent reports (3, 11, 14) that one of the important reasons that vitamin supplementation was of questionable value in our trials was that the cows in the herd were

at all times well fed and that such feeding provided vitamin reserves in the new-born calf.

SUMMARY

Individual records were kept of 299 calves of the Station dairy herd. These were divided at random into four groups to determine the value of vitamin supplementation of the ration during the first 30 days, the critical period in the life of the calf.

The vitamin supplements were of two kinds. One consisted of vitamin capsules containing vitamins A and D, together with ascorbic acid and nicotinic acid. Ascorbic acid was omitted after 10 days of age. The other was a special feeding oil containing vitamins A and D.

The criteria used in evaluating the results were gains in liveweight to 15 days and to 30 days of age, number of cases of scours, age at which scours occurred, duration of scours, number of deaths, and cause of death in those which failed to survive the 30-day period of observation.

The use of vitamin capsules containing ascorbic acid and nicotinic acid in addition to vitamins A and D was not found superior to supplementation with only vitamins A and D, and vitamin supplementation on the whole was of doubtful value, as shown by the bases of measurement used.

REFERENCES

(1) FRASER, W. J., AND BRAND, R. E. Milk Required to Raise a Dairy Calf. Ill. Agr. Expt. Sta. Bul. 164. 1913.

(2) HANSEN, R. G., PHILLIPS, P. H., AND RUPEL, I. W. The Effect of Vitamin Supplements upon Survival of New-Born Calves. Jour. Dairy Sci., 29: 761–766. 1946.

(3) HIBBS, J. W., AND KRAUSS, W. E. The Effect of Feeding Vitamin A on the Blood Picture and on Liver Storage in Calves. Jour. Dairy Sci., 29: 519–520. 1946.

(4) JOHNSON, B. C., WIESE, A. C., MITCHELL, H. H., AND NEVENS, W. B. The Metabolism of Nicotinic Acid and Its Role in the Nutrition of the Calf. Jour. Biol. Chem., 167: 729–736. 1947.

(5) LUNDQUIST, N. S., AND PHILLIPS, P. H. Further Nutritional Studies on Calf Scours. Jour. Dairy Sci., 25: 685. 1942.

(6) LUNDQUIST, N. S., AND PHILLIPS, P. H. Certain Dietary Factors Essential for the Growing Calf. Jour. Dairy Sci., 26: 1023–1030. 1943.

(7) NEVENS, W. B. Live-Weight Gains of Pasture-Fed Dairy Heifers. Jour. Dairy Sci., 27: 1011–1014. 1944.

(8) NORTON, C. L., EATON, H. D., LOOSLI, J. K., AND SPIELMAN, A. A. Controlled Experiments on the Value of Supplementary Vitamins for Young Dairy Calves. Jour. Dairy Sci., 29: 231–238. 1946.

(9) PHILLIPS, P. H.   Vitamins and Calf Diseases.   Amer. Vet. Med. Assoc. Jour., **105**: 39–40.   1944.

(10) PHILLIPS, P. H., LUNDQUIST, N. S., AND BOYER, P. D.   The Effect of Vitamin A and Certain Members of the B-Complex upon Calf Scours.   Jour. Dairy Sci., **24**: 977–982.   1941.

(11) SPIELMAN, A. A., THOMAS, J. W., LOOSLI, J. K., NORTON, C. L., AND TURK, K. L. The Placental Transmission and Fetal Storage of Vitamin A and Carotene in the Bovine.   Jour. Dairy Sci., **29**: 707–715.   1946.

(12) WIESE, A. C., JOHNSON, B. C., MITCHELL, H. H., AND NEVENS, W. B.   Synthetic Rations for the Dairy Calf.   Jour. Dairy Sci., **30**: 87–94.   1947.

(13) WIESE, A. C., JOHNSON, B. C., AND NEVENS, W. B.   Riboflavin Deficiency in the Dairy Calf.   Jour. Nutr. (in press.)

(14) WISE, G. H., CALDWELL, M. J., AND HUGHES, J. S.   The Effect of the Prepartum Diet of the Cow on the Vitamin A Reserves of Her Newborn Offspring.   Science, **103**: 616–618.   1946.

THE EFFECT OF CONTINUOUS INTRAVENOUS FEEDING OF VARIOUS SUBSTANCES UPON THE SECRETION OF MILK FAT[1,2]

A. I. MANN AND J. C. SHAW

Department of Dairy Industry, University of Connecticut and Storrs Agricultural Experiment Station, Storrs, Connecticut; and Department of Dairy Husbandry, Maryland Agricultural Experiment Station, College Park, Maryland

Kaufmann and Shaw (7) showed that carbohydrate when fed as the sole diet provides the precursors of the lower fatty acids of milk fat. As marked hypoglycemia does not in itself produce a decrease in the lower fatty acids (16), it was suggested that dietary carbohydrate acts indirectly either by exerting a sparing action on the utilization of the precursor substances by other body tissues or by being converted into the necessary precursors in the rumen by the action of microorganisms.

It appeared that the best test of the alternative hypotheses would be that of the intravenous and abomasal feeding of glucose to fasted cows and goats. Accordingly, methods were devised for the continuous intravenous and abomasal feeding of ruminants. Using these methods, a study was made of the effect of the administration of glucose and other substances upon the secretion of the lower fatty acids of milk fat.

METHODS

The approach to the problem of ascertaining the blood precursors of the lower fatty acids was similar to that employed in earlier studies (7) in which advantage was taken of the well-known fact that inanition decreases the lower fatty acid content of milk fat. As a marked decrease in the lower acids always occurs within a period of 24 hours of fasting, it was assumed that if the proper precursor or precursors were fed either intravenously or abomasally to a fasted lactating ruminant, the usual decrease in these lower acids would be retarded or prevented. Several substances were administered in this fashion and the effect upon the lower fatty acids of milk fat noted, as shown by the Reichert-Meissl value and, in some cases, the Polenske value. Iodine numbers also were obtained on the milk fat.

In order to maintain a relatively high level of the various substances in the blood and thus insure that these substances would be available in above-normal quantities for the possible synthesis of milk fat, it was deemed

advisable to administer such substances continuously for a period of 24 or more hours. The technic used for intravenous alimentation proved so satisfactory that it is described in some detail.

The technic consists of fixing a no. 8 or no. 10 rubber catheter in the subcutaneous abdominal mammary vein and connecting this catheter to a drip bottle by means of a gum rubber tube. It appeared advisable to anesthetize the cow. Accordingly, from 3 to 5 g. of nembutal were injected into the jugular vein at a rapid rate. The animal may be thrown or allowed
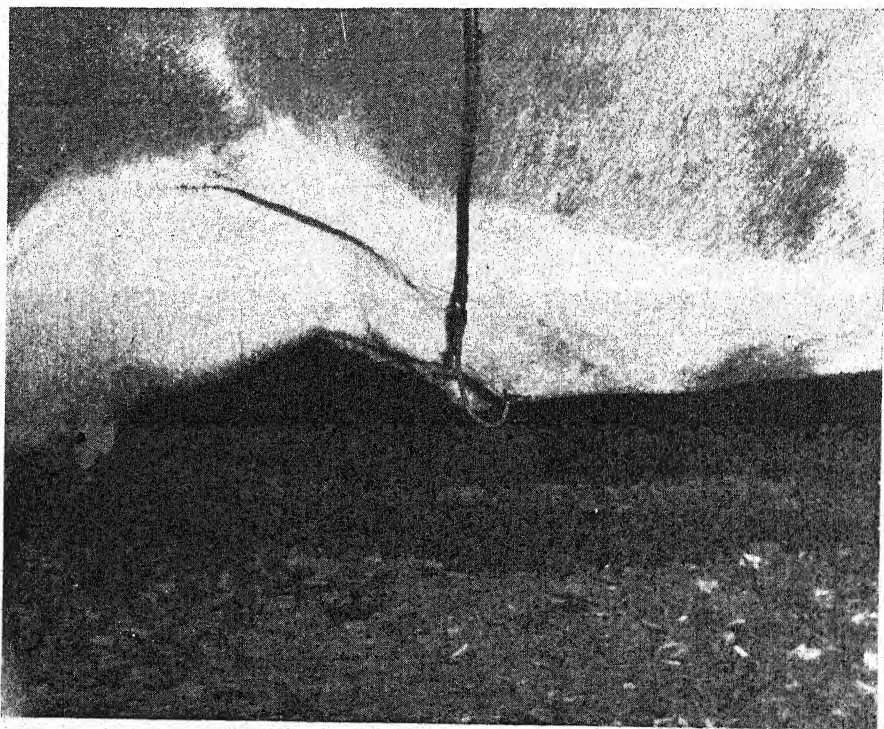
FIG. 1. The rubber catheter fixed in the vein for continuous intravenous feeding.

to fall in a well-bedded stall. It is sometimes necessary to inject an additional gram or so after the cow is in a prone position, particularly if the cow is allowed to become excited. In most cases the operation can be completed without necessitating more than one additional injection.

A small area of the skin, which has been clipped previously, is shaved and an incision approximately 3 cm. in length is made in the skin over one of the subcutaneous abdominal mammary veins. The muscles covering the vein are dissected carefully until a small area of the vein is exposed and a portion is picked up by means of a hemostat. A transverse incision is made in the vein close to the hemostat by means of a small scissors, and approxi-

mately two-thirds of the length of the catheter is inserted into the vein. If the opening in the vein is sufficiently small so that the catheter fits snugly, hematoma will be held to a minimum. Prior to beginning the operation, the clamp on the tube leading from the drip bottle is loosened so that fluid is dripping slowly from the end of the catheter when it is inserted. Three to four sutures are sufficient to close the incision. The first suture is made close to the catheter, knotted and then tied around the catheter to hold it in place. The catheter is shown in place in figure 1.

The tube leading to the drip bottle is attached to the skin by means of
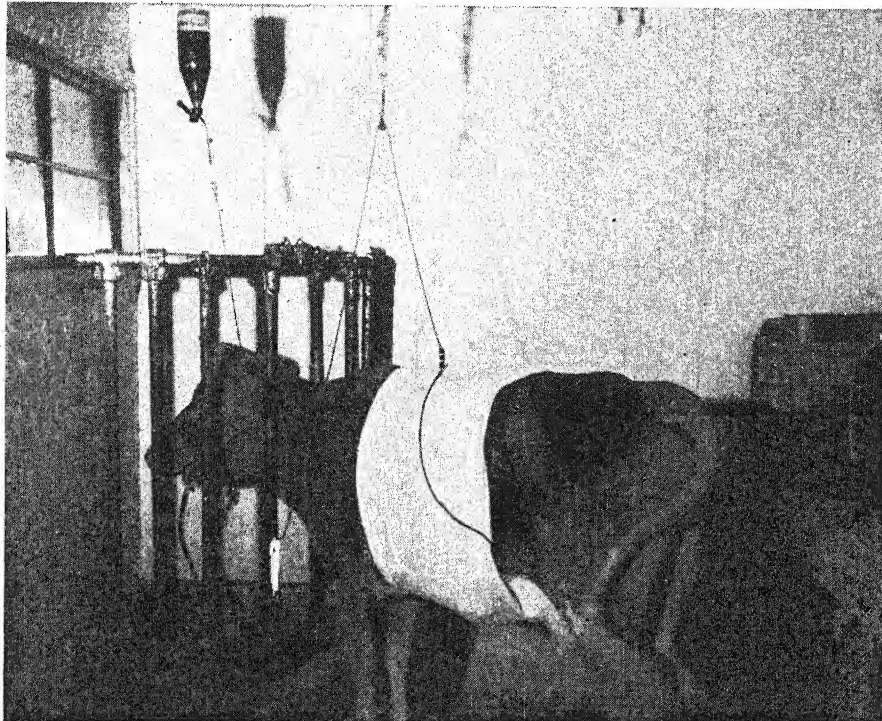
FIG. 2. The arrangement by which continuous intravenous feeding was effected without discomfort to the animal.

sutures, one in the region of the flank and the other over the last rib, approximately half the distance to the backbone. After the cow regains her feet, a wide cloth surcingle is tied around the midsection in order to cover the catheter and the tube leading to the drip bottle. The rubber tube is tied firmly to the surcingle in the region of the loin so that all pull on the tube will be on the surcingle and not on the catheter. From the surcingle the tube passes through a fixed pulley 18 to 24 inches above the backbone of the animal. A sliding weight is placed on the tube between the pulley and the drip bottle with sufficient slack so that the animal can move about and

lie down and get up at will. The details of this arrangement are shown in figure 2.

In the initial studies, goats were fed abomasally by means of a Pezzar catheter fixed in the abomasum and attached to a drip bottle in the manner described above. The abdominal cavity was opened along the ventral midline just posterior to the sternum and the catheter fixed in the abomasum by means of a purse-string suture. The catheter was brought to the exterior through an opening made between two ribs approximately halfway between the midventral line and the dorsal midline, after which the incision along the ventral midline was closed.

The iodine numbers (Hanus) and the Reichert-Meissl and Polenske values were determined on milk fat according to the methods outlined in the Official and Tentative Methods of Analysis (1).

RESULTS

Two goats in milk were fed abomasally. One received glucose and the other triacetin. Nine cows were fed by the intravenous route. The substances administered included glucose, "Pepticase"[3] (a protein hydrolysate), sodium oleate, sodium acetate, and sodium butyrate. The animals usually received grain but no roughage in the last feeding prior to the beginning of the period of intravenous or abomasal feeding.

Fat constants were determined on the purified fat prepared from milk obtained at the beginning and at the usual milking intervals during the experimental periods.

Glucose. Glucose was fed abomasally to a fasted goat and intravenously to two fasted cows, one of which received a protein hydrolysate in addition to the glucose. That the blood glucose level was maintained above normal is evidenced by the fact that in each of the three cases the glucose intake was maintained just slightly below that which produced a mild glucose shock. It is apparent, therefore, that no blood glucose deficiency existed during the fasting periods. In experiment 2 a total of 1985 g. of glucose was injected in 31 hours. It will be noted from the data on experiments 1, 2, and 3 in table 1 that neither the decrease in the Reichert-Meissl values nor the increase in the iodine values was prevented by the administration of glucose.

Protein hydrolysate. Pepticase was administered to one cow by the intravenous route to test the possibility of certain amino acids being involved in the synthesis of the lower fatty acids of milk fat. As blood glucose does not appear to be one of the precursor substances, glucose was administered with the protein hydrolysate with the object of providing additional energy so that the protein hydrolysate would not be utilized too rapidly for energy purposes. During an injection period of 31 hours, a total of 464 g. of Pepticase and 1392 g. of glucose was administered. The Reichert-Meissl value

[3] Supplied through the courtesy of Sheffield Farms Company.

TABLE 1

The effect of intravenous and/or abomasal feeding of glucose, sodium oleate, and a protein hydrolysate to fasted ruminants upon the character of the fatty acids of milk fat

Animal no.	Method of administration	Test substance	Hours after regular feeding	Grams of test substance fed (cumulative)	Milk fat constants			Comments
					R.M.V.*	P.V.†	I.N.‡	
1 (goat)	Abomasal	Glucose 15%			22.94		34.74	Normal milk
			18					Started expt. feeding
			24	325 }				
			36	496 }	20.06		39.12	
			59	775 }	17.92		43.12	
2 (cow)	Intraven.	Glucose 20%			24.44		21.95	Normal milk
			23					Started infusion
			39	855	21.49		26.82	
			51	1340	17.07		27.62	
			63	1741	16.73		29.00	
			70	1985	18.29		27.61	
3 (cow)	Intraven.	Glucose (g) 15% and "Peptidase" (p) 5%			24.83		22.08	Normal milk
			22					Started infusion
			39	558 (g) 186 (p)	20.32		28.09	
			49	870 (g) 290 (p)	16.68		27.34	
			63	1188 (g) 396 (p)	15.10		24.46	
			70	1392 (g) 464 (p)	14.99		27.37	
7 (cow)	Intraven.	Sodium oleate 5.5%			25.19	2.21	37.93	Normal milk
			17					Started infusion
			33	150	21.65	1.35	44.05	

* R.M.V. = Reichert-Meissl values.
† P.V. = Polenske values.
‡ I.N. = Iodine numbers (Hanus).

decreased gradually from 24.83 to 14.99. The iodine value increased from 22.08 to 27.37. These values are fairly representative of changes which would be expected from inanition alone.

Oleic acid. After fasting a cow for 17 hours, 150 g. of sodium oleate in a 5.5 per cent solution were injected by the intravenous route within the following 16 hours. The experiment was terminated when excessive hemoglobinuria was observed. The data presented in table 1, experiment 7, do not indicate that oleic acid had any influence in arresting the changes in the milk fat constants which are typical of fasting.

Acetic acid. Four experiments were conducted to study the possible rôle of acetic acid in the synthesis of the lower fatty acids of milk fat. In one experiment acetic acid was administered abomasally to a milking goat as triacetin. Three cows received sodium acetate intravenously, one receiving sodium butyrate with the acetate. It is evident from the experiments in which acetate was administered alone (experiments 4, 5, and 6, table 2) that the intravenous injection of acetate did not prevent the changes in the milk fat which are typical of fasting.

The amounts of acetate administered are believed to be considerably in excess of that which is normally absorbed into the circulatory system after being produced in the rumen. In experiment 6, a total of 1984 g. of acetate was injected within 22 hours. This experiment was terminated when the cow suffered a temporary collapse resembling tetany. The collapse apparently was due to an alkalosis caused by the metabolizing of the acetic acid, thus freeing an excess of sodium ions in the blood.

Butyric acid. Four cows received sodium butyrate intravenously. Two of the cows received butyrate alone during a period of complete fasting, one received a combination of butyrate and acetate while being fasted, and one received butyrate while on full feed. These animals (nos. 9, 10, 11, and 12, table 3) had been receiving a ration of mixed hay, corn silage, and a 16 per cent concentrate prior to being placed on experiment. To avoid any possible effects of inanition, all four were fed in excess of requirements for a week prior to the experiment. One of the four, no. 12, was fasted without receiving injections of any kind in order to serve as a control for the other three. The fifth cow, no. 8, had been on a regime completely different from the others.

In the first test of butyrate, the results were startling. Cow no. 8 received a total of 1057 g. of sodium butyrate during a period of 29 hours. Not only was the Reichert-Meissl value of the milk fat maintained, but the Polenske value, representing primarily the octanoic and decanoic acids, actually increased from a normal of 2.40 and 2.36 to 2.89 and 2.75. These fat samples were checked repeatedly, without any substantial change in the original values. It will be noted, however, that the iodine values increased by about the same magnitude as that of the milk fat of the control cow.

TABLE 2

The effect of intravenous and/or abomasal feeding of triacetin and sodium acetate to fasted ruminants upon the character of the fatty acids of milk fat

Animal no.	Method of administration	Test substance	Hours after last regular feeding	Grams of test substance fed (cumulative)	Milk fat constants			Comments
					R.M.V.*	P.V.†	I.N.‡	
4 (goat)	Abomasal	Triacetin 5%	...	...	22.52	......	28.86	Normal milk
			17	...	......	......	......	Started expt. feeding
			24	18	18.75	......	36.01	
			35	35	16.07	......	36.22	
5 (cow)	Intraven.	Sodium acetate 15%	...	...	19.02	......	23.92	Normal milk
			17	...	......	......	......	Started infusion
			24	165	15.64	......	33.33	
			37	333	13.32	......	33.69	
			49	668	11.99	......	38.73	
6 (cow)	Intraven.	Sodium acetate 20%	...	...	23.94	1.24	43.25	Normal milk
			19	...	......	......	......	Started infusion
			41	1984	18.57	0.88	46.14	Symptoms of alkalosis

* R.M.V. = Reichert-Meissl values.
† P.V. = Polenske values.
‡ I.N. = Iodine numbers (Hanus).

TABLE 3

The effect of intravenous feeding of sodium butyrate and sodium butyrate plus sodium acetate to cows upon the character of the fatty acids of milk fat*

Cow no.	Test substance	Hours after last regular feeding	Grams of test substance fed (cumulative)	R.M.V.†	P.V.‡	I.N.**	Comments
8	Sodium butyrate 12.6%			25.17	2.40	33.03	Normal milk
		7		25.31	2.36	32.14	Started infusion
		11½	100	26.44	2.89	34.84	
		26	589	25.05	2.75	38.21	
		36	1057				
9	Sodium butyrate 12.6%	9		31.10	3.35	33.90	Normal milk
		38	1184	25.02	2.20	42.85	Started infusion
		49½	1396	20.83	2.17	43.00	Symptoms of alkalosis
10	Sodium acetate 14% (a) plus sodium butyrate 12.6% (b)			31.44	2.81	33.55	Normal milk
		8	361 (a) 322 (b)	30.15	3.11	35.07	Started infusion
		15					
		30	1092 (a) 975 (b)	24.02	2.14	39.80	
11	Sodium butyrate 12.6% plus normal ration			28.98	3.61	27.85	Normal milk
		4		28.92	3.40	28.35	Started infusion
		11	136	29.45	3.70	26.20	
		38	786				
12	None			26.89	2.82	33.35	Normal milk
		13		26.35	3.34	36.90	Complete fasting
		37		17.61	0.92	42.65	

* Cows 9, 10, 11, and 12 received identical rations considerably in excess of requirements for one week prior to the experimental periods.
† Reichert-Meissl values.
‡ Polenske values.
** Iodine numbers (Hanus).

When the experiment was repeated on cow no. 9, the results on the Reichert-Meissl and Polenske values were not duplicated. Instead, the Reichert-Meissl and Polenske values decreased. Similar results were obtained on cow no. 10, which received 975 g. of butyrate and 1092 g. of acetate over a period of 22 hours. Cow no. 11 received a total of 786 g. of butyrate over a period of 34 hours, during which time the animal was on full feed. The butyric acid did not alter the fat constants from the normal values.

Cow no. 12, which had been on a regime identical to that of cows 9 and 10, was fasted completely for 35 hours. The decrease in the Reichert-Meissl value was similar to that observed in cows 9 and 10. The Polenske value showed a more marked change, however, decreasing from 2.82 to 0.92, whereas the fasted cows receiving butyrate decreased from 3.35 to 2.17 in one case and from 2.81 to 2.14 in the other.

DISCUSSION

With neither blood lactic acid nor blood pyruvic acid being utilized in measurable quantities by the active mammary gland of the normal cow (8, 12), the only possible blood carbohydrate which could supply sufficient carbon to account for the lower fatty acids of milk fat is that of blood glucose. The experiments described herein show that when a continuous flow of glucose is administered intravenously at the highest possible rate tolerated by the cow for a period in excess of 2 days, the decrease in the lower fatty acids of the milk fat caused by inanition is not prevented. It must be concluded that blood carbohydrate *per se* is not the precursor of the lower fatty acids of milk fat.

It appears clear that carbohydrate administered orally is not a direct precursor of the lower acids (7), but probably exerts a sparing effect or is converted into the necessary precursors in the alimentary tract.

It is known that the active ammmary gland produces urea (4) and contains arginase (14) which may be used by the gland for the deamination of amino acids. The quantities of amino acids taken up by the gland are sufficient to account for the carbon in the lower fatty acids. This appeared to be a possibility when it was shown that the active gland of the fasted ruminant did not take up the free amino acids from the blood (10, 16). However, the failure of an intravenous administration of a protein hydrolysate to prevent the decrease in the lower fatty acids of the milk of the fasted cow appears to rule out this possibility.

Sodium oleate was fed by the constant flow method to test the earlier hypothesis of Hilditch and Paul (6) and of Shaw and Petersen (15) that the lower fatty acids are derived from the degradation of the longer chain fatty acids. The results do not indicate that the lower acids are derived from oleic acid. However, the amount of oleate injected was necessarily small and definite conclusions probably are not warranted.

In view of the recent work showing that acetic acid is used in the synthesis of fatty acids, this substance appeared to offer considerable promise as a precursor of the lower fatty acids of milk fat, since it is formed in the rumen in considerable quantities. Further, such synthesis would explain the ability of food carbohydrate but not blood carbohydrate to serve as the precursor of the lower acids. However, the Reichert-Meissl and Polenske values on milk fat from fasted ruminants receiving considerable acetic acid abomasally and intravenously were typical of fasted animals.

Butyric acid, being produced in the rumen by the fermentation of carbohydrate, appeared to be another possible precursor substance. Four cows received relatively large quantities by the intravenous route. The results are conflicting. In one case there was actually an increase in the Polenske value, and the normal Reichert-Meissl value was maintained even though the cow was fasted. These results were not duplicated in succeeding experiments, although the Polenske value of the milk fat of the two additional fasted cows receiving butyrate did not decrease nearly so much as in the case of a fasted control cow. While no conclusions appear to be warranted, the data do suggest some interesting possibilities.

It may be that the butyric acid was being converted to acetone bodies and burned at such a rapid rate that little reached the mammary gland except in the one case. The same could also apply to acetic acid. On the other hand, if butyric acid was being made available to the gland in these experiments and was not being incorporated into the triglyceride, it will necessitate thinking in terms of something more complicated than a simple recombination of glycerol and fatty acids in the formation of the triglycerides of milk fat.

There has been considerable disagreement as to whether blood carbohydrate is the precursor of the lower fatty acids of milk fat. Because of the considerable amount of data which has been accumulated in the past few years in studies concerned with this possible relationship, and because of the rather conclusive negative data reported in this paper, it may be well to examine and summarize the data collected to date.

The suggestion that the lower fatty acids of milk fat are synthesized directly from carbohydrate is based entirely upon the reports that the respiratory quotient of the normal active gland of the ruminant exceeds unity (3, 9, 12) and that the respiratory quotient of the gland of the fasted ruminant is less than unity (9, 16). It is pointed out that these two findings coincide with the fact that fasting decreases the short-chain fatty acids as well as the carbohydrate content of the body. The high respiratory quotient (RQ) of the active normal gland, which was first reported by Graham et al. (3), appears to be established fairly well. The reports by Reinecke et al. (9) and by Shaw et al. (16) that the RQ of the gland of the fasted ruminant is less than unity, are based on only a few observations.

Because of the difficulty involved in obtaining representative RQ's on the gland, many more data are needed to establish the latter with certainty. In addition, Reinecke *et al.* reported that 48 hours or more of fasting were required to produce a low RQ. It is difficult to correlate this with the fact that the greatest decrease in the lower fatty acids occurs within the first 24 hours of fasting. However, assuming that the RQ of the gland of the fasted ruminant is low, what does it represent in terms of the metabolism of the gland?

In the first place, most investigators have been unwilling to accept the RQ alone on any particular organ as proof of the type of metabolism taking place within that organ. Recent work showing that carbon dioxide actually is used in synthesis within the body has tended to confirm this view. Secondly, there is considerable doubt of a true metabolic relationship between the decrease in the lower fatty acids and fasting on the one hand, and the lowering of the RQ of the gland reported for the fasted ruminant on the other, since it has not been possible to demonstrate a consistently low RQ for the glands of cows in which the lower acids had been decreased markedly by the feeding of cod-liver oil (12).

Even if it were possible to establish such a relationship, it must be kept in mind that neither the feeding of cod-liver oil nor fasting decreases the lower acids more than 35 to 40 per cent. If the lower fatty acids are synthesized from carbohydrate, how then can we account for a low RQ with 60 per cent or so of these acids still being so synthesized?

The amount of glucose available to the gland for the synthesis of fat appears to be entirely insufficient for the synthesis of the lower fatty acids. Graham first attempted to measure the blood flow in relation to glucose uptake by the gland, using the thermostromuhr method, and concluded that the glucose uptake was insufficient to account for more than 50 per cent of the milk lactose (2). At the time it was believed that the thermostromuhr method of measuring blood flow in vivo was quite accurate. However, very exhaustive experiments by Gregg *et al.* (5), Shipley *et al.* (18), and Shipley and Gregg (17) have shown that such is not the case. They obtained errors as great as 300 per cent and stated that " . . . the flow of blood in an artery of an animal can only by chance happening be determined from a unit applied to it which had been previously calibrated in an artificial circulation system . . . this is so, because, for the same unit it is indeed an accident when in vitro and in vivo environments influence the differential temperature-flow relations in the same direction and to the same extent."

By determining the calcium, inorganic phosphorus, and glucose uptake by the gland and the total amount of calcium, phosphorus, and lactose secreted during a 24-hour period, it was possible to calculate the blood flow, the total amount of glucose utilized by the gland, and the amount of glucose available for other purposes after accounting for lactose (16). Five such

experiments showed a blood flow of 494 volumes for each volume of milk formed, with a range from 408 to 561. The glucose uptake was sufficient to account for 105.5 per cent of the milk lactose. In the five experiments it was found that the glucose uptake was sufficient to account for 81.4, 101.2, 115.4, 115.3, and 97.9 per cent of the lactose secreted.

As any mammary gland balance must depend upon the accuracy of the arteriovenous determinations in representing the true metabolism of the gland, this method of approach is subject to a great deal less error than the thermostromuhr method. The accuracy of a mammary gland balance based on the thermostromuhr method is dependent upon whether the arteriovenous differences represent the true average uptake by the gland as well as the accuracy of the determination of the rate of blood flow. The large errors reported for the latter method are much greater than that which would be expected where the rate of blood flow is calculated from the uptake of calcium and phosphorus and the total amount of calcium and phosphorus secreted in the milk as shown by the actual analysis of the milk.

Data obtained by Shaw and Petersen (15) and Shaw, Boyd, and Petersen (13) can be used to make similar calculations, although in this case the average analysis of milk must be used, and the blood calcium and glucose uptake do not necessarily represent the same cow or experiment. However, the values are sufficiently numerous and uniform to obviate gross errors. Twenty arteriovenous differences for plasma calcium averaged 0.31 mg. per cent. Converting this to whole blood values on the basis of a 30 per cent cell volume, the difference becomes 0.217 mg. per cent. Assuming that the average calcium content of the milk is 120 mg. per cent, the ratio of calcium in the milk to calcium uptake becomes 553 to 1. The average of 40 determinations of the glucose utilization by the gland was 9.3 mg. per cent. At a ratio of 553 volumes of blood to one unit volume of milk, the glucose uptake could account for 5142 mg. per cent of lactose. With an average lactose content of 4900 mg. per cent in milk, this represents sufficient glucose to account for approximately 105 per cent of the lactose. The five complete balances of Shaw et al. (16) are in good agreement with these calculations. The complete balance experiments included fat. To account for the lower fatty acids in these trials, the data show that from 25 to 30 per cent of the glucose would be needed if we are to postulate that the lower fatty acids are formed from carbohydrate. Even with the small amount of glucose taken up as glyco-protein (10), it appears obvious that with neither blood lactic acid nor pyruvic acid being utilized by the gland, the gland does not remove sufficient carbohydrate from the blood to account for both lactose and the lower fatty acids.

Also opposed to the suggestion that fasting causes a decrease in the lower acids by depleting the carbohydrate available for the synthesis of these acids, is the finding that the gland of the cow with ketosis continues to remove a

normal amount of glucose from the blood even when the blood glucose falls as much as 50 per cent (11), a value much lower than would be attained by two days of fasting. Further, it was observed that even such low values were not associated with a decrease in the lower fatty acids in those cases of ketosis in which the animals retained their appetites, indicating a relationship between feed intake and the lower acids, but not between blood glucose and these acids.

Finally, with the data reported in this paper to the effect that the maintenance of the blood glucose of the fasted cow at a high level does not prevent or retard the decrease in the lower fatty acids, the evidence against the postulation that these fatty acids are synthesized in the gland from carbohydrate appears to be conclusive.

SUMMARY AND CONCLUSIONS

1. A technic was developed for the continuous intravenous feeding of ruminants.

2. The continuous intravenous injection of cows with a protein hydrolysate, glucose, oleic acid, and acetic acid failed to prevent the decrease in the lower fatty acids of milk fat caused by fasting. As much as 1984 g. of sodium acetate was administered in 22 hours and as much as 1985 g. of glucose was administered in 31 hours.

3. A summary of the work to date renders the theory of a blood carbohydrate origin of the lower fatty acids extremely unlikely.

4. The data obtained from the continuous injection of butyric acid are inconclusive.

REFERENCES

(1) *Association of Official Agricultural Chemists.* Official and Tentative Methods of Analysis. 5th Ed. Washington, D. C. 1940.

(2) GRAHAM, W. R., JR. The Utilization of Lactic Acid by the Lactating Mammary Gland. Jour. Biol. Chem., 122: 1–9. 1937.

(3) GRAHAM, W. R., JR., HOUCHIN, O. B., PETERSON, V. E., AND TURNER, C. W. The Efficiency of the Mammary Gland in the Production of Milk. Amer. Jour. Physiol., 122: 150–153. 1938.

(4) GRAHAM, W. R., JR., JONES, T. S. G., AND KAY, H. D. The Production of Urea in the Mammary Gland. Jour. Biol. Chem., 120: 29–33. 1938.

(5) GREGG, D. E., PRITCHARD, W. H., ECKSTEIN, R. W., SHIPLEY, R. E., ROTTA, A., DINGLE, J., STEEGE, T. W., AND WEARN, J. T. Observations on the Accuracy of the Thermostromuhr. Amer. Jour. Physiol., 136: 250–262. 1942.

(6) HILDITCH, T. P., AND PAUL, H. The Occurrence and Possible Significance of Some of the Minor Component Acids of Cow Milk Fat. Biochem. Jour., 30: 1905–1926. 1936.

(7) KAUFMANN, O. W., AND SHAW, J. C. The Use of Simplified Diets in the Study of the Fat Metabolism of the Mammary Gland. Jour. Dairy Sci., 28: 467–472. 1945.

(8) POWELL, R. C., JR., AND SHAW, J. C. The Non-utilization of Lactic Acid by the Lactating Mammary Gland. Jour. Biol. Chem., 146: 207–210. 1942.

(9) Reinecke, E. P., Stonecipher, W. I., and Turner, C. W.   The Relation Between
      the Fat and Carbohydrate Metabolism of Lactation, as Indicated by the Respira-
      tory Quotient of the Mammary Gland.   Amer. Jour. Physiol., 132: 535–541.
      1941.
(10) Reinecke, E. P., Williamson, M. B., and Turner, C. W.   The Utilization of
      Glycoprotein of the Blood Plasma by the Lactating Mammary Gland.   Jour.
      Biol. Chem., 138: 83–90.   1941.
(11) Shaw, J. C.   Studies on Ketosis in Dairy Cattle.   V.   The Development of Ketosis.
      Jour. Dairy Sci., 26: 1079–1090.   1943.
(12) Shaw, J. C.   Lactic Acid, Pyruvic Acid, Amino Acids, Acetone Bodies, Oxygen,
      Carbon Dioxide, and Hemoglobin in Arterial and Mammary Venous Bloods of
      Cows Under Various Physiological Conditions.   Jour. Dairy Sci., 29: 183–197.
      1946.
(13) Shaw, J. C., Boyd, W. L., and Petersen, W. E.   Blood Glucose and Lactic Acid in
      Milk Secretion.   Soc. Expt. Biol. and Med. Proc., 38: 579–584.   1938.
(14) Shaw, J. C., and Petersen, W. E.   Arginase in the Mammary Gland.   Soc. Expt.
      Biol. and Med. Proc., 38: 631–632.   1938.
(15) Shaw, J. C., and Petersen, W. E.   The Fat Metabolism of the Mammary Gland.
      Jour. Dairy Sci., 23: 1045–1056.   1940.
(16) Shaw, J. C., Powell, R. C., Jr., and Knodt, C. B.   The Fat Metabolism of the
      Mammary Gland of the Normal Cow and of the Cow in Ketosis.   Jour. Dairy
      Sci., 25: 909–921.   1942.
(17) Shipley, R. E., and Gregg, D. E.   The Effect of External Constriction of a Blood
      Vessel on Blood Flow.   Amer. Jour. Physiol., 141: 289–296.   1944.
(18) Shipley, R. E., Gregg, D. E., and Wearn, J. T.   Operative Mechanism of Some
      Errors in the Application of the Thermostromuhr Method to the Measurement
      of Blood Flow.   Amer. Jour. Physiol., 136: 263–274.   1942.

THE REMOVAL OF THE SORBED GASES IN DRIED MILKS[1]

H. S. HALLER AND G. E. HOLM

Division of Dairy Research Laboratories, Bureau of Dairy Industry,
Agricultural Research Administration, U. S. Department of Agriculture

INTRODUCTION

The results of experiments initiated in 1942 (2) on dried milks packed in air and in inert gas indicated that the keeping quality of the products packed in an inert gas atmosphere of which the final oxygen concentration was 3 to 4 per cent was considerably greater than that of the products packed in air (20.9 per cent oxygen). Although the keeping quality of dried milk depends to a great extent on the freshness of the liquid milk used and the methods employed in its desiccation, the improvement in keeping quality that was effected by reducing the oxygen concentration of the containers was so marked that recommendations were made for the consideration of this method of packing for all dried milks which had to undergo rigorous conditions of storage, especially those destined for overseas use (4). Reduction of the oxygen concentration in the commercial containers to values of less than 3 per cent did not seem practical at that time. The results of Lea, Moran, and Smith (3) published in 1943 indicated also that the keeping quality of dried milks was increased when the oxygen concentration within the dried milk containers was decreased. Subsequent studies (1, 5, 6) confirmed these observations and indicated the methods necessary to obtain oxygen concentrations of different values.

Although the practice of packaging dried milk in atmospheres of reduced oxygen concentration had been used to a limited extent for some time in the industry when this work was begun, information was lacking on the amounts of gases held in dried milks by various forces, the factors that are concerned in the removal of these gases, and the relative effect of different oxygen concentrations on keeping quality.

Spray-dried milks are composed of finely divided and very porous particles and, therefore, have enormous total surface areas (internal as well as external) per unit weight of product. Thus it seems that gases may be held by the particles by adsorption and by occlusion, and in the fat by absorption. In the following discussion these gases, however held, will be considered together under the designation of sorbed gases.

Aside from the specific characteristics of a porous material, the temperature, time, and degree of evacuation are important factors in the removal of

197

sorbed gases.   Hence, the effect of variations in these factors, as well as of the moisture content, on the amount of sorbed gases was studied to determine the relative importance of each factor and to determine the practical conditions that may be employed to remove these gases from dried milks.

APPARATUS AND ITS OPERATION

A diagram of the apparatus used throughout these studies to remove the sorbed gases, measure their volume, and determine their composition is shown in figure 1.
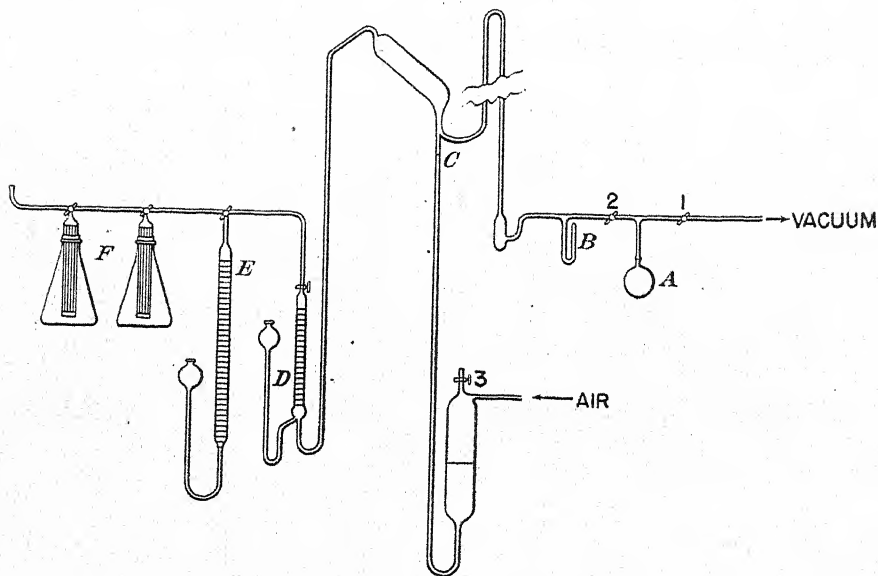
FIG. 1.   Toepler pump and gas analysis system.

A is a 500-milliliter ground-glass-joint flask that may be immersed in a constant-temperature bath.   B is a mercury manometer, C a Toepler pump, D the gas burette, E the gas-collecting chamber of the gas analysis apparatus, and F the absorption system, consisting of a carbon-dioxide absorption burette containing potassium hydroxide and an oxygen-absorption burette containing alkaline pyrogallol solution.   The manifold system is made of capillary tubing and the horizontal portion is mercury filled.   The absorption burettes and the gas burette are connected to the manifold by three-way T stopcocks.

The flask A containing the 225 g. sample was immersed in a bath held at the desired temperature.   The oil vacuum pump was then operated and the sample evacuated for the desired period of time at the desired degree of vacuum.   When the vacuum required was less than the full vacuum of the pump, it was obtained by adjusting stopcock 1 and observing the manometer B, stopcock 2 being open to the Toepler system.   After the desired vacuum

treatment, stopcocks 1 and 2 were closed and the Toepler system completely evacuated by operating stopcock 3. Stopcock 2 then was opened and the residual sorbed gas in the sample was desorbed over a period of about 6 hours by heating the sample in *A* to 70° C. (or higher) and operating the Toepler pump at intervals of about 30 minutes to remove the gas and store it in burette *D*, where it was measured. When it was desired to analyze the desorbed gas, it was transferred to burette *E*, where it was then measured both before and after absorption of the carbon dioxide and the oxygen in the appropriate solutions.

EXPERIMENTAL

In the following discussion the term "residual gas" has been used to designate the gases which can be removed from the system as indicated above after a given evacuation procedure has been used. The total amount of residual gases consists of the free gases remaining in the system plus the sorbed gases which can be removed from the product. After an evacuation at a vacuum of 3 to 5 mm. pressure, the amount of free gas in the container is but a small proportion of the total amount of residual gas. The proportion increases as the degree of evacuation decreases.

The amount of residual gas in a container varies with the efficiency of the evacuation process, *i.e.*, degree, temperature, and time of evacuation. The amount of residual gas which can be removed with a Toepler pump varies with the temperature at which the product is held, the time of evacuation, and the physical structure of the product.

At a given temperature the amount of residual gas obtained with a Toepler pump during the first interval of time is relatively large and decreases with each successive interval. After 6 hours the amount obtained at each interval is very small and practically a constant. The total amount obtained during 6 hours was considered the residual gas content of the product.

If the temperature of the product during desorption is increased, the amount of residual gas increases as indicated in figure 2.

At temperatures greater than 70° C., although larger volumes of gas are obtained, slight discoloration of the product occurs, suggesting slight decomposition. A temperature of 70° C. was therefore chosen as that at which desorptions were to be carried out.

Although the total amount of sorbed gases was not removed under these conditions, the values obtained are comparable. From data obtained it was estimated that approximately 80 per cent of the residual gas was removed in the manner prescribed, at 70° C.

The Effect of the Temperature of the Product during Evacuation upon the Amount of Residual Gas

An increase in the temperature usually decreases the amount of gas adsorbed by a porous material. Samples of dried milk were treated at a

vacuum of 10 mm. pressure for 10 minutes at temperatures of 20°, 30°, and
40° C., respectively, and the amount of residaul gas then determined as
previously described. The amounts of residual gas obtained at 30° and
40° C. were slightly but not significantly less in each case than the amount
obtained at 20° C. However, when short periods of evacuation and products
of moisture contents greater than usual are concerned, the efficiency of
evacuation will be affected by the vapor pressure of the product, this pres-
sure increasing with temperature. Under these conditions the efficiency of
the process may be less at the higher temperatures. One-half-pound samples
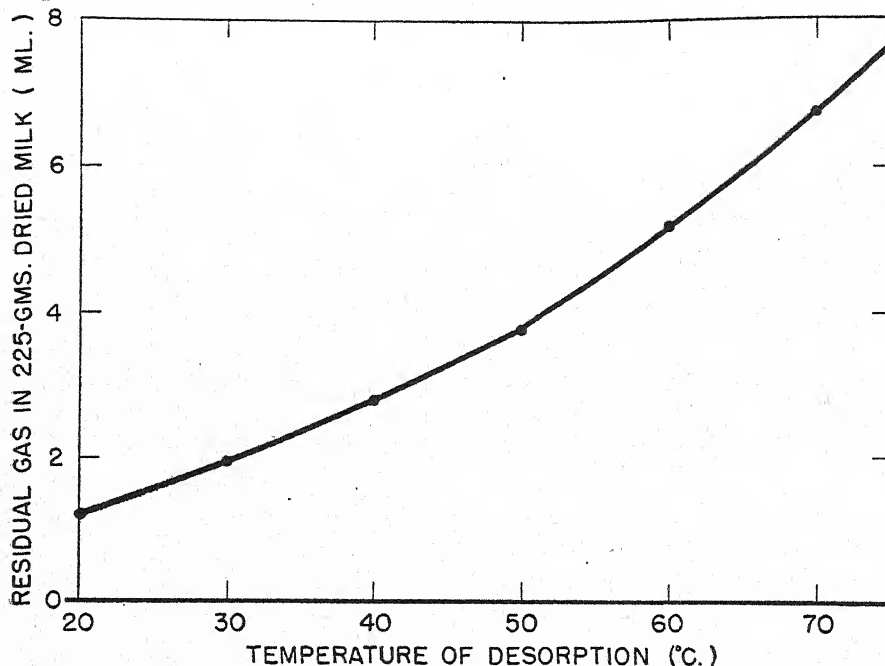
Fig. 2. Amounts of gas obtained at different temperatures of desorption.

of a commercial dried milk were subjected to the vacuum of an oil pump for
3 minutes at 20°, 30°, 40°, and 50° C., respectively, and the actual vacuum
attained in the system was noted. The values are shown in figure 3. Under
similar conditions of evacuation, a number of tins of dried milk were pack-
aged in nitrogen. After storage of 3 days at room temperature, the gases
in the head space of the cans were analyzed. The average oxygen percent-
ages of the gas in the cans evacuated at the different temperatures are shown
in figure 4. It is evident that the values obtained are those which can be
obtained only under the conditions used. They will differ with the moisture
content of the product, the time of evacuation, the capacity of the pump
used, and other factors. However, the results do emphasize the fact that

increasing the temperature at which the product is held does not necessarily increase the amount of gas removed by evacuation.

The Effect of the Time of Evacuation upon the Amount of Residual Gas

It is logical to assume that the longer a dried milk is held under a vacuum the less will be its residual gas content.
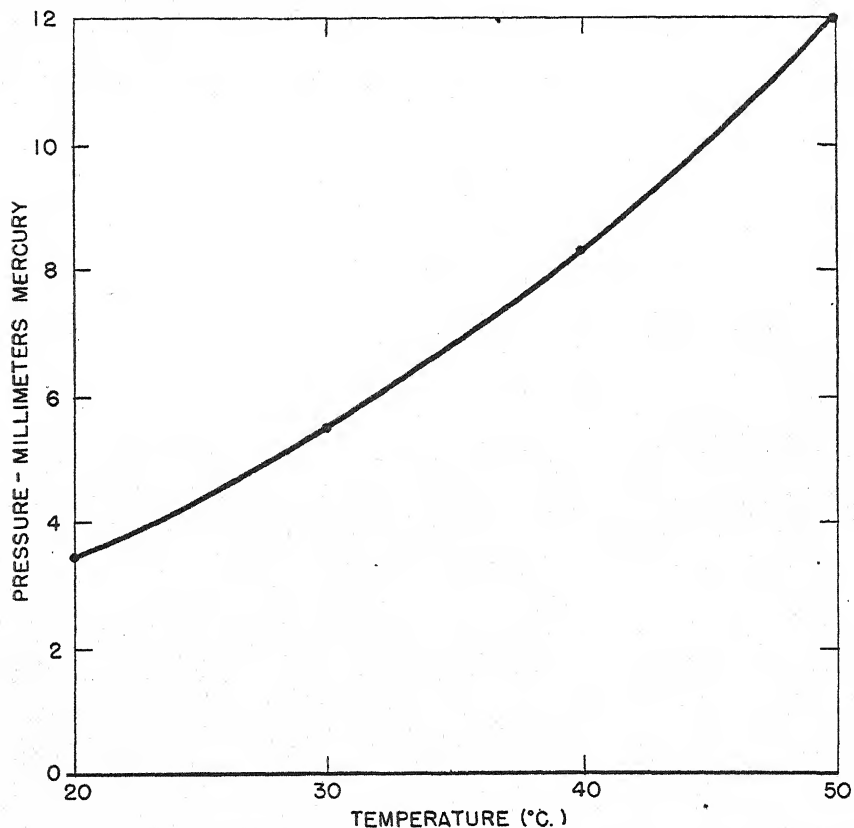
FIG. 3. Manometric pressure obtained by evacuation of dried milk for 3 minutes at different temperatures.

Figure 5 shows the results on an old sample of dried milk after treatment at various degrees of vacuum at room temperature. The values shown indicate that from 15 to 30 minutes is required to evacuate dried milks to a point where additional evacuation will produce only a small regular decrease with increase in pumping time. As expected, the amount removed increases with the time of evacuation, and increases more at the higher degrees of evacuation.
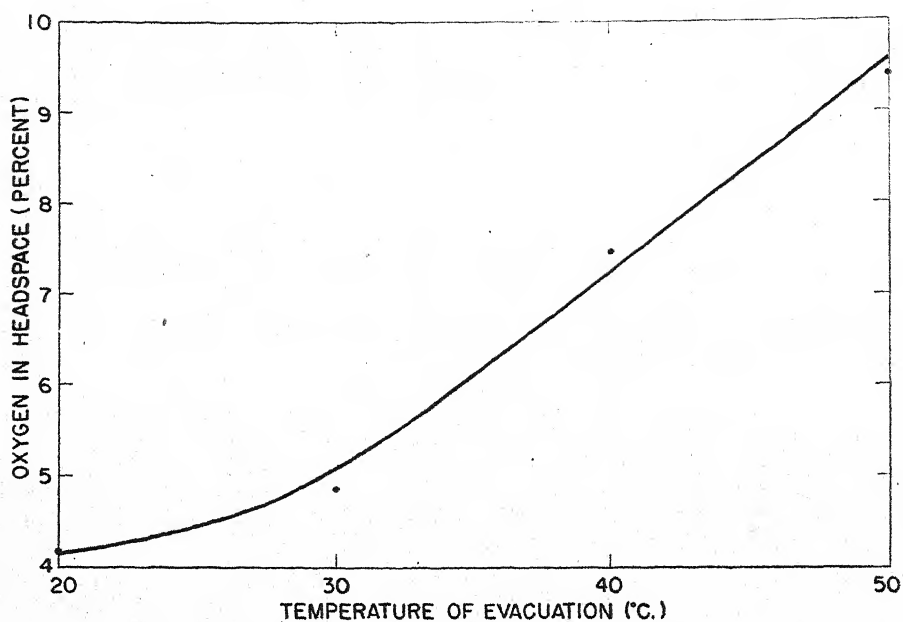
FIG. 4. Oxygen percentage in dried milk containers evacuated for 3 minutes at various temperatures.
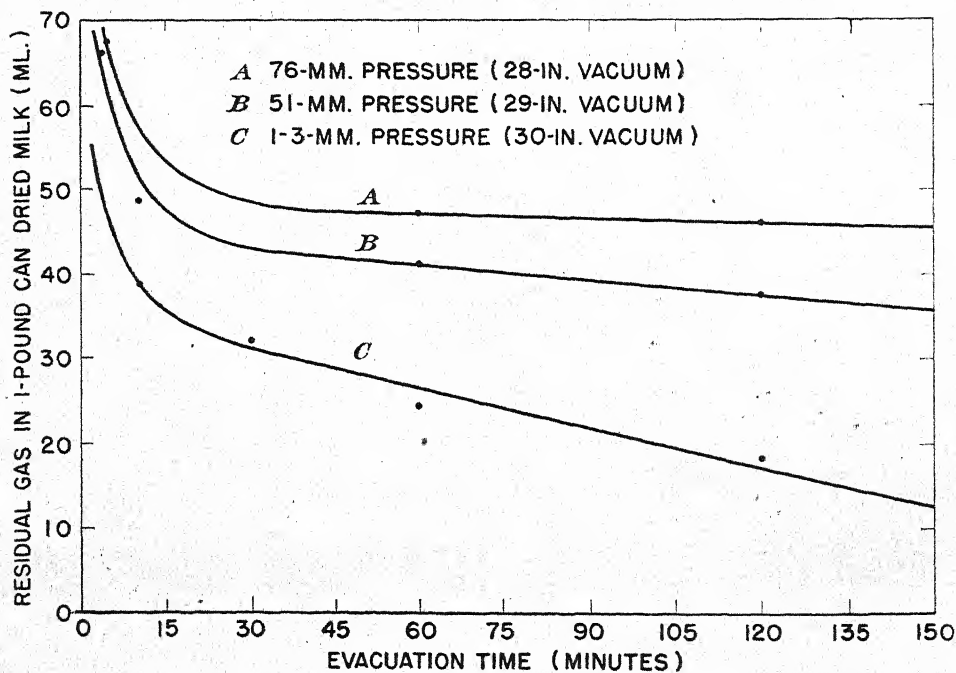
FIG. 5. Amounts of residual gas obtained from dried milks evacuated at different degrees of vacuum for different periods of time.

Effect of the Degree of Vacuum Used upon the Amount of Residual Gas

The time of evacuation used in these experiments was 10 minutes, the temperature 25° C., and the degree of vacuum 10 mm. pressure of mercury. The results are given in figure 6.

It is apparent from these data that the amount of sorbed gas decreases but slightly as the degree of vacuum increases from 51 to 3 mm. of pressure. The amounts of gas removed (B) at 25° C. represent the gas in the free space of the container after evacuation under the given conditions. The
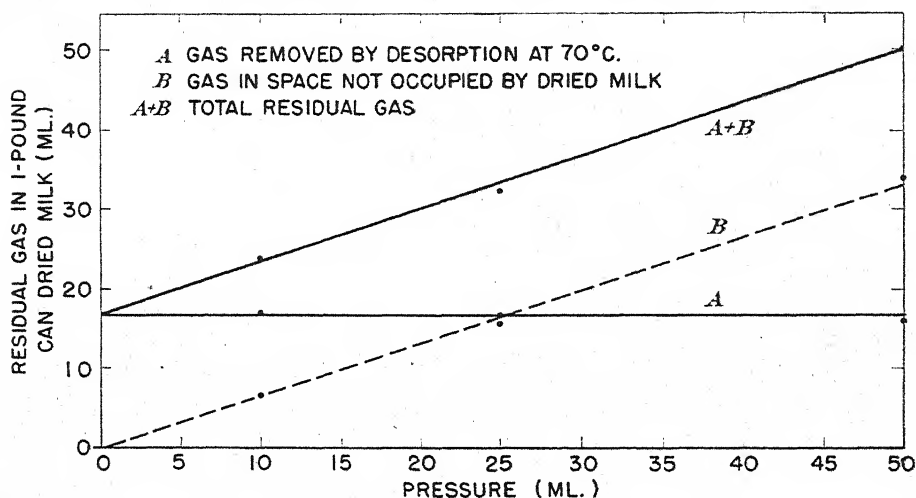
FIG. 6. Amounts of residual gas in dried milks evacuated at different degrees of vacuum.

values represented by A are the amounts of sorbed gas obtained when the temperature was increased to 70° C. The total amounts of residual gas are represented by A + B.

From the data presented it is evident that increases in time, temperature, and pressure of evacuation beyond practical values do not decrease greatly the amount of the sorbed gases held by the product. However, the desirability of the use of as complete a vacuum as possible is indicated in figure 6. At vacuums of several millimeters pressure, the amount of gas in the free space is, of course, very small. At 25 mm. of pressure it is approximately 17.50 ml. or 3.65 per cent of the free space in a 1-lb. container and is approximately equal to the amount of residual gas in the dried milk which can be removed by heating the product to 70° C. Upon evacuation at a vacuum of 51 mm. pressure, the amount of gas in the free space is approximately twice that of the residual gas.

Effect of the Concentration of the Milk upon the Residual Gas of Its Dried Product

It had been noted in preliminary experiments that dried milks made by different methods and of different degrees of fineness differed greatly in the amounts of residual sorbed gases. Samples of dried milk therefore were prepared from milks of different degrees of concentration varying from
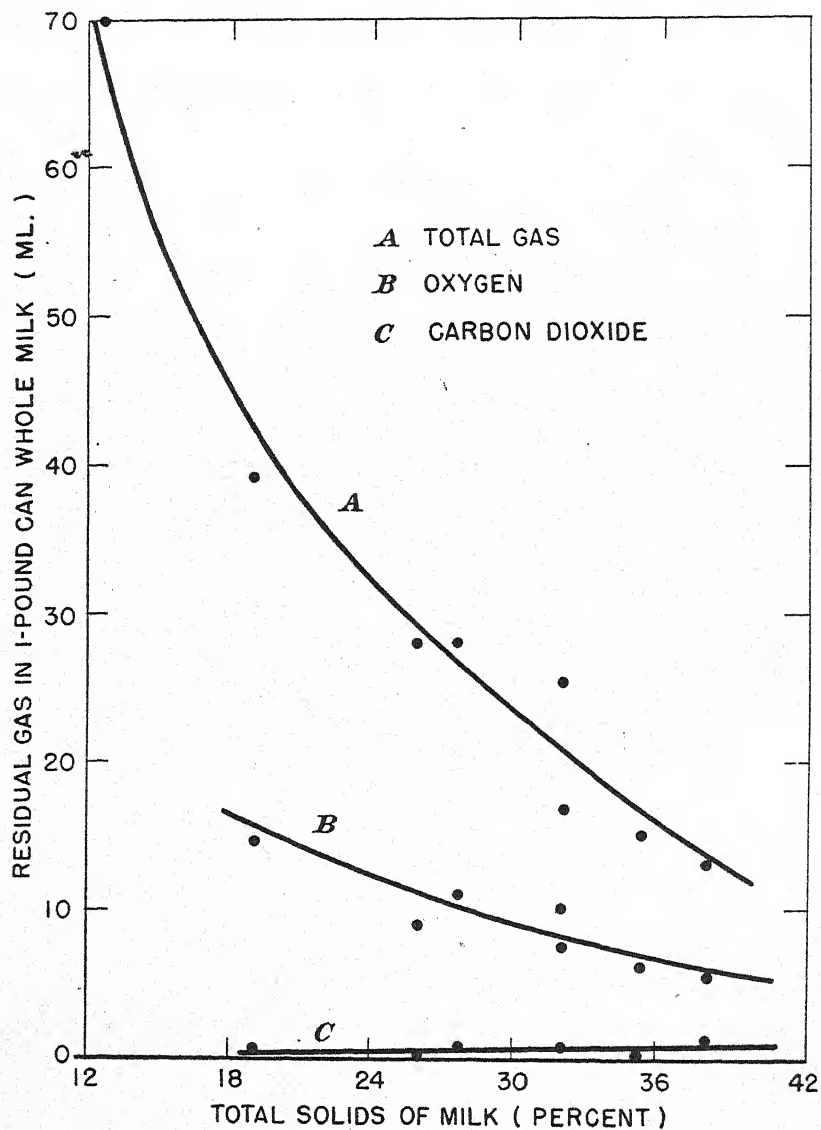
FIG. 7. Residual gas in dried milks prepared from milks of different concentration.

13 per cent to 38 per cent solids. The product made from milk of 13 per cent solids was finely divided and of low apparent density. As the concentration of the milk increased, the dried milks became coarser in texture and of greater apparent density. The results on determinations of residual gases in these samples are given in figure 7.

The amounts of residual gas, or gas which remained in the products after treatment at full vacuum of an oil pump for 1 hour at 25° C., decreased rapidly with increases in the solids concentration of the milk used. The decrease in the amounts of oxygen was relatively smaller.

Effect of Moisture Content upon the Amount of Sorbed Gases

A series of experiments was conducted to determine the effect of variations in the moisture content on the amount of sorbed gases remaining in dried milks after vacuum treatment. Samples of two dried milks containing less than 2 per cent of moisture were allowed to absorb moisture from relatively humid atmospheres until the desired moisture content was obtained
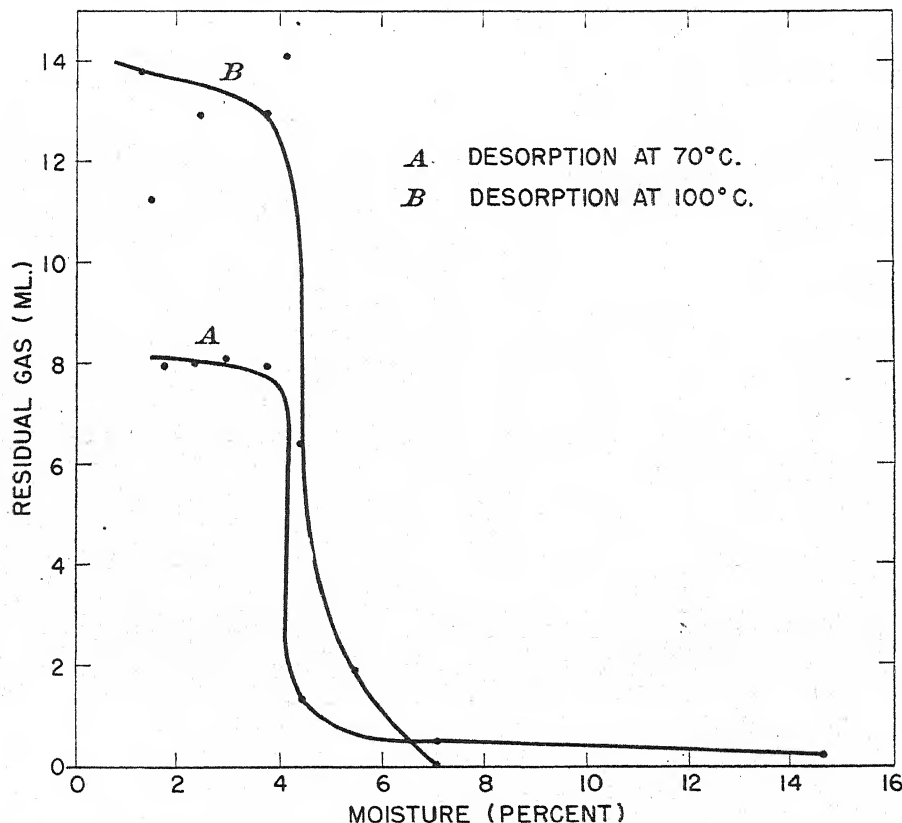
FIG. 8. The residual gas in dried milks of different moisture percentage.

in each case. Samples ranging in moisture content from the original amount to over 7 per cent were obtained in this manner. Each sample was subjected in turn to the full vacuum of an oil pump for 1 hour at 25° C., with an anhydrous magnesium perchlorate desiccant inserted between the sample and the remainder of the system. The residual gas of each sample was then determined with the temperature of the sample maintained at 70° C. The results are shown in figure 8.

Within the range of moisture content of commercial dried milks the amount of sorbed gases differed but slightly with differences in the moisture content. However, if the products absorbed moisture to amounts greater than 4 per cent, there was an abrupt decrease in the amount of sorbed gases. Whether the absorption of moisture facilitates in some manner the removal of gases during evacuation or whether the moisture is adsorbed preferentially has not been determined.

Diffusion of Sorbed Gases

To what extent the sorbed gases can be removed by the removal of nitrogen into which these gases have been allowed to diffuse for a period of time was determined.

TABLE 1

Percentage composition of gas in container after successive evacuations, filling with nitrogen, and storing for different periods of time

. Period	Nitrogen	Oxygen	Carbon dioxide
	(%)	(%)	(%)
1st (3 days)	97.8	2.2	Trace
2nd (3 days)	99.6	0.4	Trace
3rd (4 days)	99.8	0.2	Trace
4th (3 days)	100.0	Trace	Trace
5th (4 days)	100.0	Trace	Trace

A freshly made sample of 225 g. of dried milk quickly was brought to full vacuum with the oil and Toepler pumps. Nitrogen then was bled into the system until the system was brought to atmospheric pressure; the sample was allowed to remain under these conditions for 3 days. A sample of the gas in the system then was withdrawn and analyzed, and the system again evacuated and filled with nitrogen. This cycle of procedure was repeated until the percentage of oxygen content of the nitrogen admitted was practically zero. The results are shown in table 1.

After five stages of evacuation the system containing the sample was evacuated quickly again with the oil and Toepler pumps to rid it completely of free gases. The sample container then was immersed in a bath maintained at 70° C. and the liberated gases were removed with a Toepler pump and analyzed.

The amount obtained was 6.15 ml., which is equivalent to 70.1 per cent

of the amount of sorbed gases that were removed from another sample of the same milk under similar conditions, except that no diffusion was allowed to occur. The composition of the residual gas obtained after the five successive evacuation and diffusion periods was as follows: Nitrogen, 95.9 per cent; oxygen, 4.1 per cent; carbon dioxide, trace. Hence, it is indicated that although all of the oxygen cannot be removed except by continued exhaustive removal by desorption and evacuation extending over a long period of time, the amount of oxygen which remains in the residual gas after two cycles of this process is relatively small.

Percentage Composition of the Residual Gas

Aside from the amounts of gas retained by the dried milks after evacuation, the oxygen concentration in this gas is of interest. In table 2 are shown the results of analyses of the residual gases from two freshly made and three relatively old samples of dried milk.

TABLE 2

Composition of residual gases from representative samples of dried milk

No.	Description of sample	Oxygen	Nitrogen	Carbon dioxide
		(%)	(%)	(%)
1	Freshly made. Evac. 29.6 in., 10 min., at 25° C. ...	32.7	68.9	1.4
2	Freshly made. Evac. 29.6 in., 15 min., at 25° C. ...	39.0	61.0	0.0
3	Old sample. Evac. 30 in., 5 min., at 25° C.	25.6	65.0	9.4
4	Same as no. 3. Evac. 30 in., 10 min., at 25° C.	22.0	68.9	9.1
5	Same as no. 3. Evac. 30 in., 45 min., at 25° C.	23.7	66.1	10.2

The values in table 2 indicate that the amount of residual oxygen is relatively high and that of carbon dioxide is practically nil in freshly made dried milk. In aged samples the proportion of oxygen is less and the carbon dioxide considerably more than in freshly made dried milks. Also, the proportion of carbon dioxide in the residual gas is considerably more than would be expected if only the partial pressures of this gas in the atmosphere were concerned. However, carbon dioxide is adsorbed more strongly than the other gases concerned and therefore seems to concentrate in the product with a resulting displacement of other gases, in this case evidently oxygen.

These results support the belief that adsorption is a vital factor in the retention of gases by dried milks. If occlusion alone were concerned, the percentage composition of the gases would be that of the air used in the drying procedure and also would not vary greatly with the age of the product. The release of oxygen by the product with age is of interest. To what

extent it can enter into consideration in practical procedures requires further study.

DISCUSSION AND SUMMARY

The amount of sorbed (occluded, adsorbed, and dissolved) gases in dried milks varies greatly with the fineness of the product. Dried milks made from milks of normal concentration contain a relatively large amount of sorbed gas, which decreases greatly as the concentration of the milk used is increased from 9 to 38 per cent solids.

A large percentage of the sorbed gases can be removed by evacuation for relatively short periods of time within the practical range of temperatures of 20° to 40° C. The remainder only can be desorbed very slowly as the time of evacuation is extended.

The composition of the sorbed gas varies with the storage time of the dried milk in an atmosphere of air. Freshly made products seem to have percentage concentrations of oxygen greater than air and very low percentage concentrations of carbon dioxide. In older products, the proportion of oxygen in the sorbed gases is but slightly greater than that in air and the proportion of carbon dioxide is greater than can be accounted for if the gases are occluded air.

The results indicate that most of the residual gases are held by adsorption forces.

A large proportion of the oxygen of the sorbed gas may be removed by successive evacuations, with periods of several days between to allow for diffusion of the oxygen into a nitrogen atmosphere. The results indicate that two cycles of evacuation—filling with nitrogen, and holding for 3 to 4 days—remove a high percentage of the oxygen of the sorbed gases.

REFERENCES

(1) COULTER, S. T., AND JENNESS, R. Packing Dry Whole Milk in Inert Gas. Minn. Agr. Expt. Sta. Tech. Bul. 167. 1945.

(2) GREENBANK, G. R., WRIGHT, P. A., DEYSHER, E. F., AND HOLM, G. E. The Keeping Quality of Samples of Commercially Dried Milk Packed in Air and in Inert Gas. Jour. Dairy Sci., 29: 55–61. 1946.

(3) LEA, C. H., MORAN, T., AND SMITH, J. A. B. The Gas-Packing and Storage of Milk Powder. Jour. Dairy Res., 13: 162–215. 1943.

(4) QMC Subsistence Research and Development Laboratory Conference on the Packaging of Dried Milks, New York City, June 29–30, 1943.

(5) SCHAFFER, P. S., GREENBANK, G. R., AND HOLM, G. E. The Rate of Autoxidation of Milk Fat in Atmospheres of Different Oxygen Concentration. Jour. Dairy Sci., 29: 145–150. 1946.

(6) SCHAFFER, P. S., AND HOLM, G. E. The Oxygen Content of the Atmosphere in Containers of Dried Milk Packaged in Nitrogen. Jour. Dairy Sci., 29: 207–212. 1946.

ASSOCIATION ANNOUNCEMENTS

The Annual Meeting of the American Dairy Science Association will be held at Ontario Agricultural College, Guelph, Ontario, June 24, 25, and 26, 1947. Further information concerning the meetings will appear in later issues of the JOURNAL.

D. M. Seath, chairman of the Production Section, has appointed the following men to the Committee on Dairy Cattle Judging Contests: S. M. Salisbury, Ohio, *Chairman;* D. L. Fourt, Idaho; and R. E. Johnson, Connecticut.

The membership of the Honors Committee, as listed on page 66 of the January issue of the JOURNAL, was incorrect. The members of this committee are: A. C. Dahlberg, New York, *Chairman;* A. C. Ragsdale, Missouri; and J. A. Nelson, Montana.
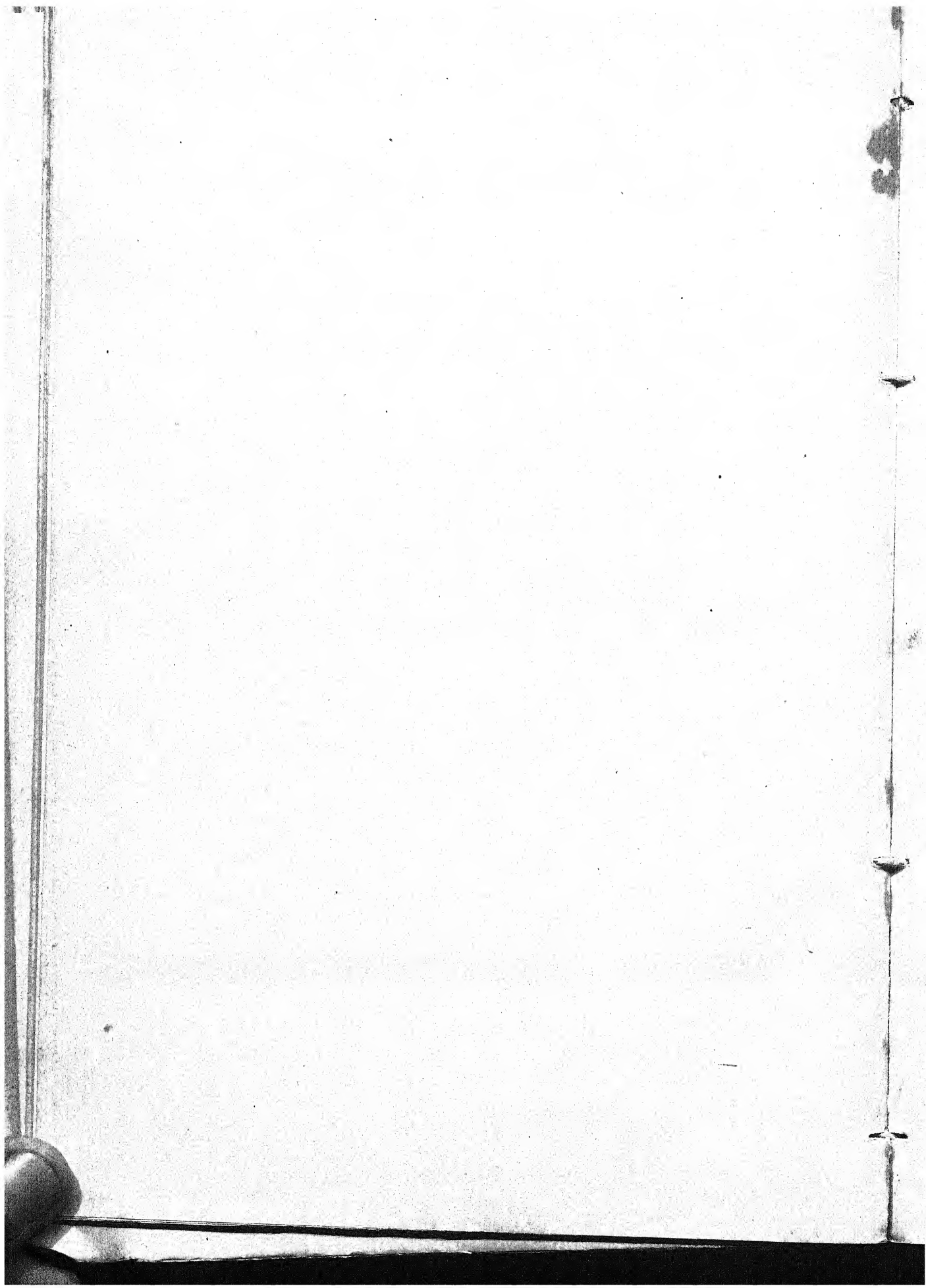
THE ACID DEGREE, FREE VOLATILE FATTY ACIDS, AND THE FLAVOR SCORE OF SALTED COMMERCIAL BUTTERS[1]

F. V. KOSIKOWSKY, A. C. DAHLBERG, E. S. GUTHRIE, AND GEORGES KNAYSI

Department of Dairy Industry, Cornell University, Ithaca, New York

It is well known that butter deteriorates during long storage as a result of oxidative or hydrolytic processes with a subsequent formation of various chemical substances. The extent of the deterioration is governed by such factors as the past history of the butter and the conditions of storage. A number of chemical changes occur in butter as a result of storage. An interesting example of these changes is found in the work of Browne (2) who noted that a sample of butter exposed to the air at room temperature for a period of about 25 years markedly increased in the content of free acids and total volatile acids and decreased in the unsaturated insoluble acid content.

Various attempts have been made to use the acid degree and the free volatile acidity of butterfat as a means of following the quality change in butter. Ferris, Redfield, and North (3) found that after keeping sweet cream butter in cold storage 5 to 6 months there was a lowering in the score of one point, while the free volatile fatty acid value was about doubled. After 6 to 7 months the butter was removed from cold storage and kept at 15° C. for 2 weeks, causing a drop in score but no increase in volatile acids. Bendixen (1), working with the acid number of fresh butter before and after storage, noted that an increase in the acid ratio (fat acidity : butter acidity) during a week at 21° C. and during 1 month at 0 to 5° C. seemed to be closely related to poor keeping quality, especially in the case of sweet cream butter. In an extensive study dealing with butter, Fouts (4) reported that most samples of unsalted butter increased in acid number of the fat during holding for 6 days at 21° C.

The relationship, however, between the acid degree, free volatile acidity, and the quality of commercial butters covering the normal flavor score has never been clearly established. Fouts (4), using butter obtained for the most part from a student scoring contest and generally scoring 90 or over,

could find little or no correlation between the acid degree and score of salted commercial butter. On the other hand, Knaysi and Guthrie (11) developed a colorimetric method for determining quality of commercial butter based on the principle that an indirect relationship existed between quality of commercial butters and concentration of free fatty acids. Guthrie (8) later stated that values obtained on a large number of butters by this method and by the method involving titration of the fatty acids correlated closely with each other, and both agreed with the flavor scores in the great majority of cases.

The possible existence of a relationship between free volatile fatty acids and the quality of commercial butter apparently requires further investigation. An attempt by Fouts (6) to study the relationship between degree of rancidity and volatile fatty acidity was limited to 14 samples of unsalted butter, all of which were rancid to some degree. It was concluded that no correlation existed between degree of rancidity and volatile fatty acid concentration.

It would appear that if a relationship exists between fat acidity and quality of experimental butter kept in storage, as indicated by Bendixen (1), a similar relationship, possibly modified to some degree, might be shown to exist with commercial market butter.

Most of the market butter today is made from pasteurized cream. As the pasteurization process inactivates lipolytic enzymes of milk and cream, there is much less chance that hydrolytic rancidity will occur. However, poor quality butters, even if manufactured from pasteurized cream, may have a high free fatty acid content as a result of certain factors. The cream prior to pasteurization may have developed some rancidity. Although some of the volatile fatty acid contributing to this rancidity would be washed out of the butter, Ferris, Redfield, and North (3) have shown that more remained in the butter from cream of high volatile acidity than in butter from cream showing low volatile acidity. In a similar way, Jack, Tarassuk, and Scaramalla (10) have obtained data which show that as the acid degree increased the flavor score of butter made from rancid cream decreased. Certain microorganisms can hydrolyze butter-fat. If the cream is improperly pasteurized or if recontamination after pasteurization occurs, these organisms may be present and eventually increase the free fatty acid content of the butter. Finally, the production of free fatty acids through oxidative rancidity should be considered.

As there was on hand a new method considered sensitive enough to determine the free volatile fatty acid content of butter over the normal marketable flavor score, a study was undertaken to investigate the possible relationship between free volatile fatty acid concentration and flavor score of salted commercial butters. At the same time, determinations of acid degree and Knaysi-Guthrie number (11) were conducted on the same butters to see to

what degree these constants were interrelated with each other and with flavor scores of salted commercial butter.

EXPERIMENTAL METHODS

Butter samples for this study were obtained in 1-lb. lots, either directly from grocery stores throughout the central New York State area or from various plants manufacturing butter throughout the state. At first the plan was to obtain all samples of butter from different groceries, but due to extreme shortages less than half of the samples could be obtained in this manner. The buttermaking concerns were instructed to send a variety of grades of marketable butter which was held by them in storage. A total of 69 samples of salted butter was obtained.

As the samples of butter were received they were placed in a cold storage room until ready to be tested, which was usually 2 or 3 days later. To prepare the butters for analytical testing, each sample had its outer layer (0.25 inch thick) removed and then some of the interior portion was melted to a liquid state in a water bath having a maximum temperature of 50° C. The melted butter was next centrifuged for a few minutes and the oil layer drawn off by means of a suction pump into a clean flask. The serum also was saved for analyses.

Analytical methods used in this work included the following:

Acid degree. The total fat acidity was determined by the recently published method of Herrington and Krukovsky (9). In this method 5 g. of butter oil were placed in a small flask, followed by the addition of 20 ml. neutral alcohol and 5 drops of 1 per cent phenolphthalein indicator (1 per cent in 50 per cent alcohol solution). The solution was heated to boiling and titrated with N/20 NaOH in 50 per cent alcohol. The results were reported as ml. N alkali required to neutralize 100 g. of fat. Acid degree and acid number were considered synonymous in this work.

Knaysi-Guthrie number. This technic gives an index of free fatty acids. The method was applied to butter by Knaysi and Guthrie (11) to estimate quality. To 1 ml. of clear butter oil in a test tube, 3 ml. of neutral red dye (pure xylol saturated with the base of neutral red) were added and the mixture well agitated. The tube was compared to a set of color standards containing known quantities of oleic acid.

Free volatile fatty acids in butter oil. Twenty grams of melted butter oil were mixed with 175 ml. of ethyl ether and the whole placed in a separatory funnel. The ether-fat solution was next washed six times with N/10 NaOH in a manner described by Gould and Johnson (7). The alkali washings carefully were heated over a hot plate to remove any ether; then they were placed in a Kjeldahl flask (800 ml.) to which 35 g. of $MgSO_4 \cdot 7 H_2O$ and a few glass beads already had been added. The mixture was brought to pH 2 with 50 per cent H_2SO_4, refluxed for 5 minutes, and distilled in

a slightly modified Kjeldahl distillation apparatus recently described by Smiley, Kosikowsky, and Dahlberg (12). Distillation continued until crystallization occurred. The distillate and neutral alcohol rinse of the condenser tube were titrated with N/20 NaOH. Both water-soluble and water-insoluble volatile fatty acid values were recorded.

Free volatile fatty acids in butter serum. The recovery of the volatile acids in the serum was conducted as follows: Ten grams of serum were added directly to a Kjeldahl flask containing 35 g. of $MgSO_4 \cdot 7 H_2O$, a few glass beads, and 280 ml. of distilled water. Enough 50 per cent H_2SO_4 was added to lower the pH of the mixture to 2. After refluxing for 5 minutes and rinsing down the refluxer with 15 ml. of distilled H_2O, the mixture was distilled. The distillate (285 ml.) with the neutral alcohol rinsings from the distillation apparatus was titrated with N/20 alkali. Water-soluble and water-insoluble acids were measured.

Total free volatile fatty acids in butter. In order to arrive at the total free volatile fatty acid content of the butter, it was assumed that for all butters the serum constituted one fifth of the butter. On this basis, the total free volatile fatty acidity of the butter was calculated on a ratio of four parts butter oil to one part butter serum. In routine analysis the tests would be made on the butter oil and serum from a weighed sample of butter.

Butter score. The butter was graded by two of the authors. Butters were scored for flavor only, but were marked on the basis of 93 to < 85 or from excellent to very poor. Butters scoring lower than 85 were considered to be unmarketable.

EXPERIMENTAL RESULTS

Since the acid degree and the Knaysi-Guthrie number deal only with butter oil, it was necessary for comparative purposes to obtain the total and water-soluble volatile fatty acids of the butter oil. Furthermore, since it was felt that the key to certain relationships might be associated with the volatile acids of the serum, this portion of the butter also was analyzed. The results obtained from the oil and serum made it possible to arrive at the total and water-soluble volatile fatty acid content of the butters.

The data obtained on 69 samples of salted butter are outlined in table 1. In this table the data are averaged together on the basis of half-point divisions in score. This type of grouping has the disadvantage of uneven weighting, as the groups vary considerably in the number of butters. In order to remedy this condition, a smaller number of groups, based on approximately equal quantities of butter (except in the last division), is presented in table 2.

It may be noted in tables 1 and 2 that inverse general relationships of varying degrees exist between butter scores and the free fatty acid concentration of the butter and butter oil expressed as acid degree, volatile acidity, and Knaysi-Guthrie number. The most clear-cut and consistent relationship existed between total volatile fatty acid and flavor score of butter. The aver-

TABLE 1

Relationship between flavor score, acid degree, and free volatile fatty acidity
of salted commercial butter

Butter score	No. of butters	Volatile acidity of butter		Acid degree	Knaysi-Guthrie no.	Volatile acidity of butter oil	
		Total	Water-soluble			Water-soluble	Total
		(ml. N/20 acid/20 g.)	*(ml. N/20 acid/20 g.)*			*(ml. N/20 acid/20 g.)*	*(ml. N/20 acid/20 g.)*
93.0	17	0.97	0.64	0.65	0.21	0.55	0.87
92.5	5	1.15	0.71	0.66	0.60	0.62	1.05
92.0	5	1.31	0.83	0.79	1.00	0.59	1.03
91.5	2	1.27	0.80	0.55	1.00	0.67	1.08
91.0	5	1.51	1.02	1.10	0.90	0.78	1.20
90.5	3	1.40	0.82	0.98	1.00	0.75	1.17
90.0	8	1.81	1.24	1.20	1.00	0.95	1.43
89.5	3	2.03	1.38	1.83	1.83	1.18	1.87
89.0	9	2.07	1.43	1.37	0.89	1.01	1.54
88.5	5	2.33	1.57	1.89	0.90	1.07	1.72
88.0	2	2.83	2.08	0.61	1.00	1.89	2.33
86.5	2	5.08	3.43	3.35	3.30	1.85	2.22
< 85.0	3	7.33	6.04	6.82	9.33	5.66	6.94

age volatile acid titer of 17 butters scoring 93.0 was 0.97 ml. N/20 acid/20 g. butter, and for 16 butters scoring 89 to 88 the average total volatile acid titer was 2.25 ml. N/20 acid/20 g. (table 2). Also, a fairly good inverse relationship was shown between the free water-soluble volatile fatty acid content and the flavor score of butter.

The data in tables 1 and 2 also reveal that the acid degree and flavor score of butter appear to be related to some extent, but the relationship was not as consistent as that shown by the volatile acids of the butters.

TABLE 2

Relationship between flavor score, acid degree, and free volatile fatty acidity
of salted commercial butter, using relatively even groupings

Butter score	No. of butters	Volatile acidity of butter		Acid degree	Knaysi-Guthrie no.	Volatile acidity of butter oil	
		Total	Water-soluble			Water-soluble	Total
		(ml. N/20 acid/20 g.)	*(ml. N/20 acid/20 g.)*			*(ml. N/20 acid/20 g.)*	*(ml. N/20 acid/20 g.)*
93.0	17	0.97	0.64	0.65	0.21	0.55	0.87
92.5 to 91.0	17	1.32	0.85	0.82	0.85	0.66	1.09
90.5 to 89.5	14	1.77	1.18	1.29	1.18	0.96	1.47
89.0 to 88.0	16	2.25	1.56	1.44	0.91	1.14	1.70
< 86.5 to no score	5	6.43	5.00	5.43	6.92	4.14	5.05

In general, based only on averaged values, as the butter quality became extremely poor, the Knaysi-Guthrie number showed a high value, while for the butter of excellent quality the Knaysi-Guthrie number showed a low value. However, in the area between excellent and extremely poor, the Knaysi-Guthrie number was irregular and insensitive.

Analysis of the data obtained with the butter oil in comparison to those
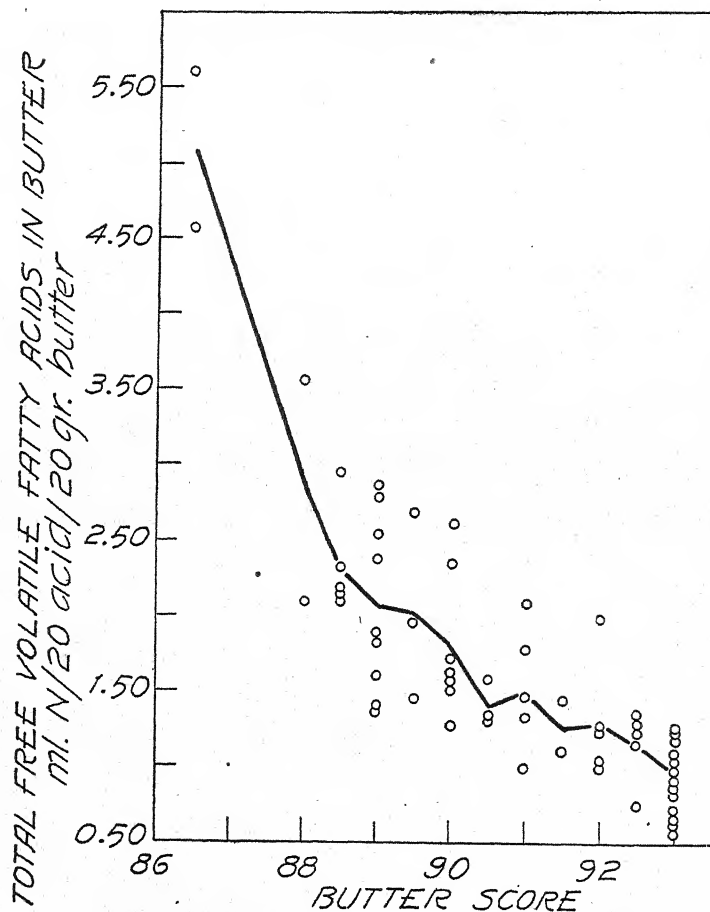
FIG. 1. The relationship between the total free volatile fatty acid content and the flavor score of salted commercial butter.

obtained on butter (tables 1 and 2) reveals that the free volatile fatty acid content of the oil exhibits a similar, but less consistent, trend in its relationship to flavor score of the butter. This indicates that the free volatile fatty acids of the butter serum, in conjunction with those of the fat, must be considered in any study of this nature if the results are to be correlated with observations on the butter.

Although data shown in tables 1 and 2 appear on the whole to exhibit clear-cut relationships in some cases, it must be emphasized that these data represent averages and not individual values and, in most instances, there was a wide range of values within each flavor score division. This is to be expected in a study of this type where many factors cannot be controlled. Some butters may be scored down as a result of defects not associated with increased fatty acids. In the case of other butters, neutralized cream may
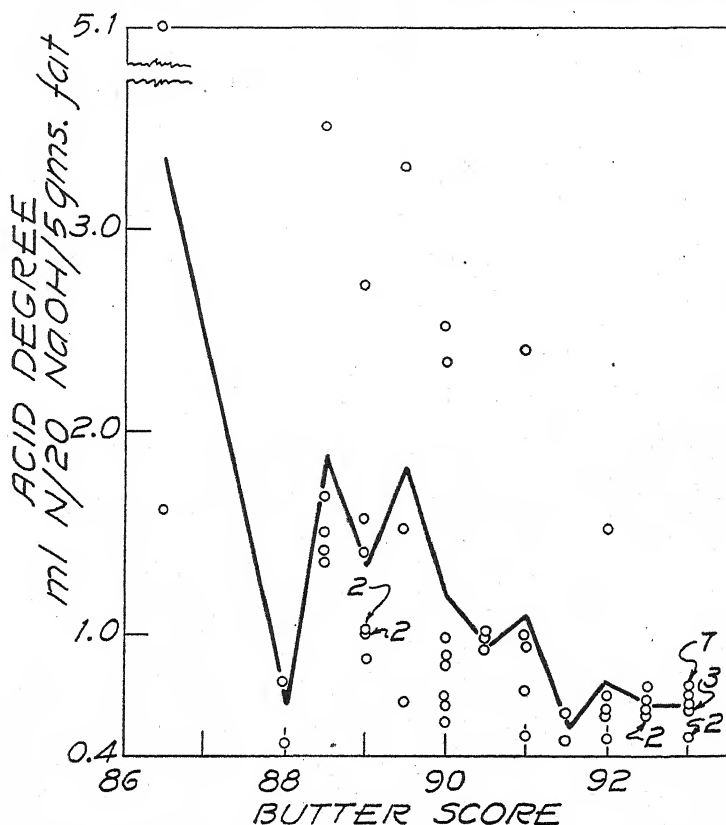
FIG. 2. The relationship between the acid degree and the flavor score of salted commercial butter. (The numbers in the figure show the times that the same data were obtained.)

produce an effect on acid degree determinations unrelated to concentration of free fatty acids. To show this point more clearly, part of the averaged data in table 1 was plotted on graphs, accompanied by results for the individual butters. Figures 1, 2, and 3 show the relationship existing, both in terms of averaged and individual values, between flavor score and total free volatile fatty acid content of butter; flavor score and acid degree; and flavor score and Knaysi-Guthrie number, respectively. Butter scores ranging from 93 to 86.5 were plotted on these graphs.

It may be seen (fig. 1) that a definite inverse relationship does exist between the free volatile fatty acids and the flavor score of salted commercial butters. However, even this relationship, though definite in trend, is quite general when individual samples of butter are considered. The acid degree plotted against flavor score (fig. 2) shows wide variations between individual samples, although the general trend is for a good butter to have a low acid
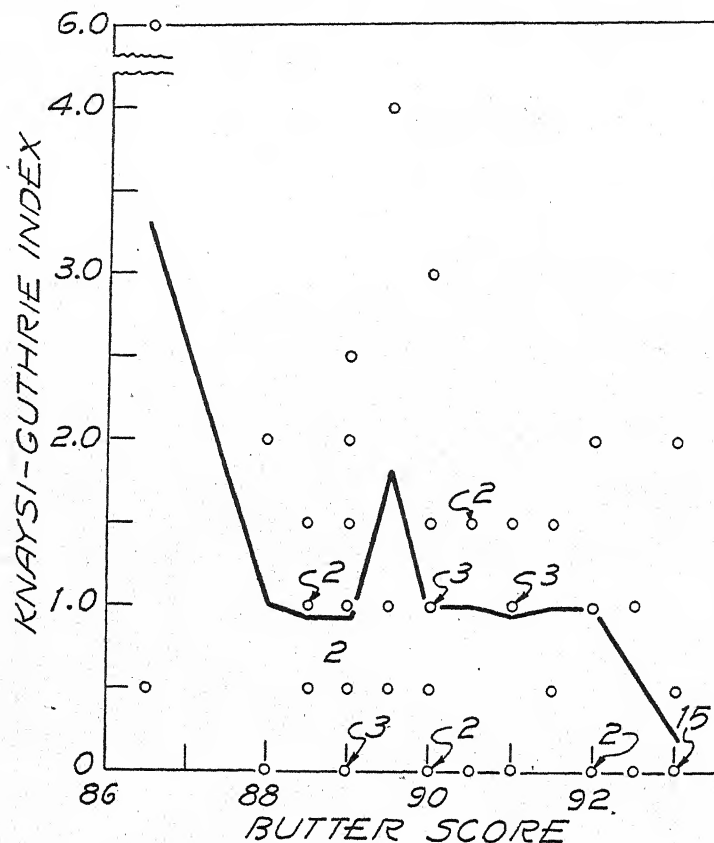
FIG. 3. The relationship between the Knaysi-Guthrie number and the flavor score of salted commercial butter. (The numbers in the figure show the times that the same data were obtained.)

degree and a poor butter a high acid degree. The relationship of Knaysi-Guthrie number to flavor score of butter (fig. 3) generally was similar to that exhibited by the acid degree.

DISCUSSION

A study was undertaken to clarify the situation in regard to the free fatty acid content of salted commercial butters and its relationship to flavor score.

Data obtained in this study tend to confirm, in part, the conclusion of Fouts (4, 6) in regard to acid degree, since only an inverse relationship of a very general nature was observed from an analysis of 69 salted commercial butters. This relationship may be disturbed by various factors. Fouts (5) found that when the titratable acidity of sour cream was reduced by the addition of an alkali, the acid number of the fat also was reduced but not proportionately. In the present investigation, several low-scoring samples of butter possessing extremely high concentrations of free volatile fatty acids showed surprisingly low acid degrees. Another factor which might upset this relationship would be the grading down of a butter because it had absorbed flavors from its environment or because it had other flavor defects not associated with fatty acids. Of 69 samples analyzed, only two of the butters were considered as having slight absorbed flavors. Naturally, the variation in the number of these types of butter would affect any relationship proportionately. However, this kind of butter probably represents only a very small percentage of the whole.

Studies on the free volatile fatty acids of butter show that these acids are allied with flavor deterioration to some extent. This appears logical, since it is well known that lower chain fatty acids, such as butyric, caproic, and caprylic, usually are associated with odors of a rancid nature in butter.

The new method evidently recovered more volatile fatty acids from butter than previous methods, and this was an important factor in showing the relationship between free volatile acids and butter score more clearly than heretofore. For example, Ferris *et al.* (3) found that the average free volatile acidity for 14 samples of 93–94 score butters was 0.65 N/10 acid per 100 g. of butter, whereas by the method used for this work, 17 samples of butter scoring 93 averaged 2.43 ml. N/10 acid per 100 g. of butter, an increase of more than three times. In this respect, Fouts (6), using a steam distillation method, found that for a series of butters exhibiting slight to pronounced rancid flavor, the percentage of total free fatty acid that was volatile ranged from 11.4 to 16.7 per cent. In contrast, from sweet-cream butters used in the current work, an average percentage recovery of approximately 30 per cent was obtained for all butter oils, again indicating more complete recovery. This value of 30 per cent was in good agreement with that obtained by Gould and Johnson (7) on fresh fat churned from milk and ether-extracted before steam distillation.

Neutralization of sour cream for buttermaking should not affect the free volatile acid value of the butter in the same way it affects the acid degree. This is due to the fact that in the process of recovery of the free volatile fatty acids, the salts of the fatty acids formed by a neutralizer would be split by acidification to pH 2. However, neutralization might be a factor in reducing some of the free volatile acids of butter as the result of increased possibilities of losses of the neutralized acid during the washing of the butter. The indication is that this effect could not be great.

In regard to the free volatile fatty acids in the serum, it was assumed that the butter existed in a ratio of four parts of butter oil to one part of serum. Actually this ratio is not always found, but for commercial butters it will remain quite constant. It is evident that for routine determination of total volatile acidity in butter, the analysis should be made on the fat and serum from a given weighed sample.

Although the free volatile fatty acids show an inverse relationship to flavor score of butter, this relationship does not appear exact enough to warrant using it as a basis for determining the flavor score of butter. As a basis for roughly determining the excellent, good, or poor qualities of a butter, it might be acceptable.

The Knaysi-Guthrie number did not correlate well with the butter score because of its apparent lack of sensitivity in regions of low fatty acid concentration. In the method advocated by Knaysi and Guthrie (11) for butter, the authors stress the fact that no perfect agreement should be expected between the quality of butter as estimated by their method and the score of the expert. The data presented herein show that 94 per cent of all butters gave Knaysi-Guthrie numbers of 2 or less and flavor scores ranging from 86 to 93, which substantiates the conclusion of Knaysi and Guthrie (11) who stated that "a test of 2 or below indicates, almost always, butter of fair, good, or excellent quality".

SUMMARY

The existence of a possible relationship between butter quality and free fatty acid concentration, including those of a volatile nature, was investigated. Methods involved included the Knaysi-Guthrie method on butterfat, a titration method for acid degree, and a modified ether-extraction direct-distillation technic for free volatile fatty acid concentration in butter and butterfat.

Free volatile fatty acid concentration of butter determined either as total or water-soluble acidity was found, in general, to be inversely related to the flavor score of 69 lots of salted commercial butter. Individual samples varied appreciably from the averages.

The relationship between the acid degree and the butter score was not well defined. An inverse relationship was discernible but individual samples varied greatly.

It was not possible to observe the existence of a close relationship between the flavor score of commercial butter and the Knaysi-Guthrie number of butterfat, even in average values, except that very high-scoring butters generally gave low values.

On the basis of the data obtained, it would not be advisable to recommend using either the free total acidity or the free volatile fatty acid values of butter as an index for determining flavor scores of salted commercial butters,

due chiefly to variation of individual samples from the averages. For a rough classification of commercial butters as excellent, good, fair, or poor, the use of free volatile acid values might have merit. However, the accumulation of much more data would be required before even this rough classification could be established.

REFERENCES

(1) BENDIXEN, H. A. Acid Values and Acid Ratios as Related to the Keeping Quality of Salted Butter. Jour. Dairy Sci., 23: 275–284. 1940.

(2) BROWNE, C. A. The Spontaneous Decomposition of Butter Fat. Indus. and Engin. Chem., 17: 44–47. 1925.

(3) FERRIS, L. W., REDFIELD, H. W., AND NORTH, W. R. The Volatile Acids and the Volatile Oxidizable Substances of Cream and Experimental Butter. Jour. Dairy Sci., 4: 521–535. 1921.

(4) FOUTS, E. L. Relationship of Acid Number Variations to the Qualities and Flavor Defects of Commercial Butter. Jour. Dairy Sci., 23: 173–179. 1940.

(5) FOUTS, E. L. Some Factors Responsible for Variations in the Acid Numbers of the Fat in Cream and in Commercial Butter. Jour. Dairy Sci., 23: 245–258. 1940.

(6) FOUTS, E. L. Relation of Volatile Acidity of Butter Fat to Rancidity. Jour. Dairy Sci., 23: 307–314. 1940.

(7) GOULD, I. A., AND JOHNSON, B. C. Solubility and Volatility of Fatty Acids Involved in Lipolysis in Homogenized Raw Milk. Jour. Dairy Sci., 27: 173–180. 1944.

(8) GUTHRIE, E. S. Butter Studies: Knaysi Method vs. Titration of Fatty Acids in Butter. Jour. Dairy Sci., 27: 678. 1944.

(9) HERRINGTON, B. L., AND KRUKOVSKY, V. N. Lipase Action in Normal Milk. Jour. Dairy Sci., 22: 127–135. 1939.

(10) JACK, E. L., TARASSUK, N. P., AND SCARAMALLA, E. L. Effect of Different Pasteurization Temperatures on Keeping Quality of Butter Made from Cream Containing Naturally Active Lipase. Natl. Butter and Cheese Jour., 33(12): 16–20. Dec., 1942.

(11) KNAYSI, GEORGES, AND GUTHRIE, E. S. A Colorimetric Method for Estimating the Quality of Butter—a Preliminary Report. Jour. Dairy Sci., 25: 589–593. 1942.

(12) SMILEY, K. L., KOSIKOWSKY, F. V., AND DAHLBERG, A. C. A Simplified Extraction-Distillation Method for the Determination of the Volatile Fatty Acids of Cheese. Jour. Dairy Sci., 29: 207–215. 1946.

THE VALUE OF GROUND WHOLE GRAINS VERSUS BY-PRODUCTS IN CONCENTRATE MIXTURES FOR DAIRY COWS[1]

C. L. NORTON[2] AND E. S. SAVAGE[3]

Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

The nature of concentrate mixtures fed to dairy cows varies widely, depending upon the availability of ingredients. In regions where farm-grown grains are raised abundantly, the concentrate mixture consists primarily of corn, oats, or barley, and a protein supplement. Other areas depend to a greater extent on various by-product feeds to supply a part of the concentrate portion of the dairy ration. Regardless of the source of ingredients, widely different mixtures give satisfactory results under practical conditions. Consequently, it was of interest to learn the relative value of quite different types of concentrate mixtures when fed under similar conditions. Two mixtures were chosen, one composed largely of by-product feeds, and another containing a high proportion of farm-grown grains.

Several investigations (1, 2, 3, 4, 5, 6, 7, 8) have been conducted comparing the nutritive value of simple and complex concentrate mixtures. In general, the results have indicated that simple mixtures are practically equal to more complex mixtures. However, little research has been conducted to compare by-product feeds and farm-grown grains when composing a major portion of the concentrate mixture. The following series of experiments was designed primarily to study the effect on milk production of concentrate mixtures composed of feeds of different sources.

EXPERIMENT A

This investigation was a double reversal feeding trial consisting of three 7-week periods. Two groups of six cows each were used. These groups were equalized on the basis of breed, age, weight, stage of lactation, and average production during a 2-week preliminary period. There were four Holsteins, one Brown Swiss, and one Ayrshire in each group. During the sixth week of the experiment, one Holstein cow died of an internal hemorrhage so her mate in the other group also was removed from the experiment.

Group I was fed the Cornell test mixture especially developed for feeding cows on official test and containing 58 per cent of by-product feeds. Group II received a ground whole grains mixture containing 76 per cent of farm-

[2] Present address: Rhode Island State College, Kingston.

[3] Deceased.

grown grains. At the end of each 7-week experimental period both groups of cows were reversed without a transitional feeding period.

The formulas of the concentrate mixtures are shown in table 1. The analyses shown in the table are for the mixtures fed in experiment C, which was conducted a year later than experiments A and B. Although the same formulas were used in all three feeding trials, the analyses showed the Cornell test mixture fed in experiments A and B contained 20.0 per cent of total protein and 5.8 per cent of fat and was calculated to contain 16.2 per cent of digestible protein and 77.8 per cent of total digestible nutrients. The

TABLE 1

Formulas and analyses of the concentrate mixtures used

Ingredients	Cornell test mixture	Check 22% mixture	Ground whole grains mixture	By-products mixture
	(lbs.)	(lbs.)	(lbs.)	(lbs.)
Ground yellow corn	340	370	500	....
Ground oats	370	300	600	....
Linseed meal	200	240	350	....
Wheat bran	360	200	....	370
Distillers' corn grains, dried	300	300	....	300
Coconut oil meal	300	240	....	....
Ground wheat	....	....	200	....
Ground soybeans	....	....	220	....
Corn gluten feed	....	....	....	400
Hominy feed	....	....	....	500
Soybean oil meal, 41% protein	....	220	....	300
Molasses	100	100	100	100
Dicalcium phosphate	15	15	15	15
Ground limestone	5	5	5	5
Salt	10	10	10	10
Total amount, lbs.	2,000	2,000	2,000	2,000
Total protein, %	18.47	22.15	17.77	22.90
Fat, %	5.27	5.18	5.44	5.58
Digestible protein, %	14.93	18.06	14.86	18.40
T.D.N., %	76.89	77.48	78.71	79.96

ground whole grains mixture fed in the first two experiments contained 20.3 per cent of total protein and 5.5 per cent of fat and was calculated to contain 16.9 per cent of digestible protein and 76.9 per cent of total digestible nutrients.

U. S. no. 2 clover-timothy mixed hay that analyzed 13.1 per cent of total protein and fairly well-eared corn silage supplied the roughage part of the ration. Hay was fed at the rate of 1 per cent and silage at the rate of 3 per cent of initial body weight. The concentrate mixtures were adjusted at weekly intervals in accordance with production the previous week. Concentrates were fed three times daily at the rate of 1 lb. per 3.5 lbs. of 4 per cent fat-corrected milk (F.C.M.). The experimental animals were milked three times a day.
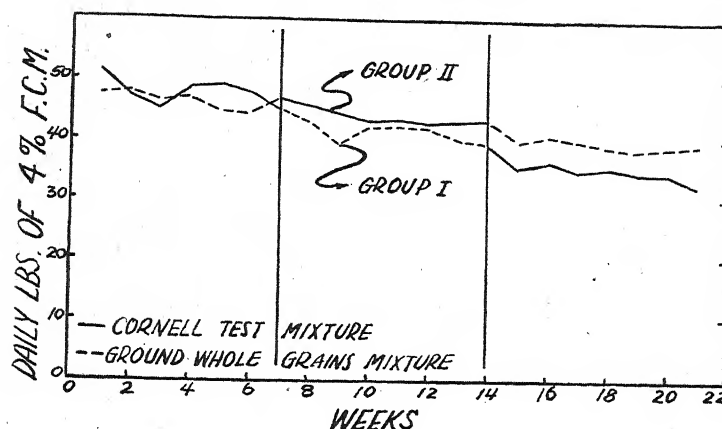
FIG. 1. The trend in milk production of the two experimental groups when receiving different concentrate mixtures.

Figure 1 shows the trend in milk production. There was very little difference between the two concentrate mixtures in promoting milk production. The average production of both groups on the Cornell test mixture shown in table 2 was 42.7 lbs. of 4 per cent F.C.M. and 42.3 lbs. of 4 per cent F.C.M. on the ground whole grains mixture. The difference in production of the groups on the two concentrate mixtures was not significant statistically. Since the difference was less than 0.5 lb. of milk per cow per day, it is likewise of little practical importance.

There was an average total gain in body weight for both groups of 3 lbs. per cow when the Cornell test mixture was fed, and an average total loss of 7 lbs. per cow during the periods when the ground whole grains mixture was fed. There was little difference in the consumption of concentrates, hay, and silage on the two rations.

From these results it appears that both concentrate mixtures were of nearly equal value when compared on the basis of milk production and maintenance of body weight. The difference in palatability, if any, was too small to be brought out by this experiment. Both mixtures were palatable enough

TABLE 2

Daily production of 4 per cent fat-corrected milk by experimental groups

	No. of cows	Cornell test mixture (control)	Ground whole grains mixture (experimental)
		(lbs.)	(lbs.)
Experiment A Double reversal trial (21 weeks)	10	42.7	42.3
Experiment B Continuous trial (26 weeks)	8	37.4	35.1

that the cows consumed all of the concentrates that were offered throughout the experiment.

EXPERIMENT B

A continuous feeding trial with two groups of six cows each was designed to study any accumulative effects of the two concentrate mixtures used in experiment A. This study was conducted simultaneously with the first experiment and extended over a period of 26 weeks. During the trial one cow in each group had to be dropped from the experiment, so the mate of each of these cows also was removed.

The experiment was summarized using only the four cows that completed the feeding trial in each group. The cows received the same general treatment and were fed at the same rates as those in experiment A. As this was a continuous trial, group III received the Cornell test mixture (table 1) for the entire 26 weeks, and group IV received the ground whole grains mixture for the same period. The trend in milk production is shown in figure 2.
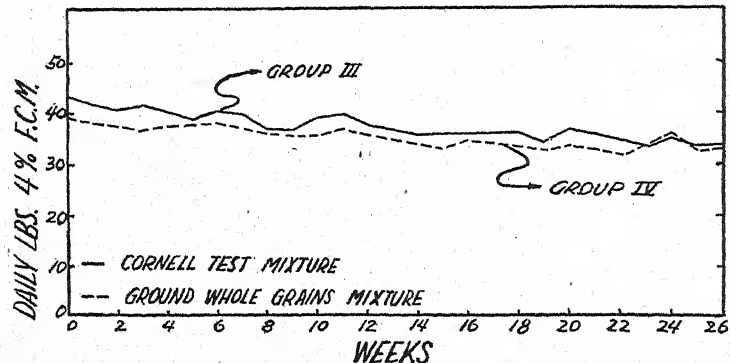
FIG. 2. The trend in milk production of the two experimental groups during a continuous feeding trial of 26 weeks.

During the experimental period, group III produced an average of 37.4 lbs. of 4 per cent F.C.M., while group IV averaged 35.1 lbs. of 4 per cent F.C.M. per day (table 2). This difference of 2.3 lbs. in favor of the group receiving the Cornell test mixture does not indicate necessarily any superiority of that mixture in feeding value. One more accurately might assign this difference in production to the fact that group III, which received the Cornell test mixture, was a higher-producing group. During the 2-week preliminary period, this group averaged 2.8 lbs. more milk than did group IV. Figure 2 shows that the trend in milk production was essentially the same. The difference existing between the groups at the end of the feeding trial was slightly less than at the beginning of the experiment. The body weight changes were of greater magnitude but were in the reverse order of those that occurred in experiment A. The cows in group III lost an average

of 22 lbs. during the 26 weeks while receiving the Cornell test mixture, and group IV showed an average gain in body weight of 12 lbs. per cow.

EXPERIMENT C

Four groups of six cows each were selected for this reversal type of experiment. Three Holsteins, one Brown Swiss, and two Jerseys were in each group. Four concentrate mixtures were fed in rotation to each group of cows in order to eliminate the effect of differences in production among the groups. The allotment of the cows to the various groups was based on breed, age, weight, stage of lactation, and expected productive ability.

The formulas and analyses of the concentrate mixtures fed are given in table 1. The Cornell test mixture and the ground whole grains mixture had been used earlier in experiments A and B. Two additional mixtures which contained approximately 22 per cent of total protein also were included. The by-products mixture consisted entirely of by-product feeds and minerals and contained 22.9 per cent of total protein. The check 22 per cent mixture was fed as a control mixture to determine the effect, if any, of a slightly higher protein mixture, since the two mixtures first mentioned contained about 18 per cent of total protein.

These two latter mixtures contained a larger amount of protein for two practical reasons. First, mixtures of such protein content fit the type of roughage fed on many farms, especially where non-leguminous roughages are fed. Second, it is difficult to make up a satisfactory mixture of by-products alone that has less than 20 per cent of total protein.

The average grade of the hay fed was no. 2 timothy medium clover mixed hay, and this hay contained 10.2 per cent of total protein. The corn silage was of excellent quality and was fairly well-eared.

The experimental periods were 6 weeks in length. At the end of each period the concentrate mixtures were changed abruptly with no intervening transition. The cows were milked three times daily, and the concentrates were fed previous to each milking. Hay and silage were fed twice daily. Concentrates were fed at the rate of 1 lb. for each 3.5 lbs. of 4 per cent F.C.M. produced daily during the previous week. One pound of hay was fed for each 100 lbs. of body weight. Slightly more than 3 lbs. of corn silage per day per 100 lbs. of body weight were fed. Daily weighbacks of uneaten hay were recorded, but it was unnecessary to take weighbacks of concentrates or silage.

It was somewhat surprising that the concentrate mixtures that differed so much in their ingredients were eaten so readily when the cows were changed abruptly from one mixture to another. Judging from the rate at which the cows consumed the mixtures, it must be assumed that they were unable to detect or were indifferent to the overnight changes that occurred at the end of each experimental period. There were no observable differences in the palatability of the four concentrate mixtures.

Table 3 gives a summary of the production of 4 per cent F.C.M. This table summarizes the production on the basis of concentrate mixtures and groups of cows. The highest average production, 42.9 lbs. of 4 per cent F.C.M. per cow per day, was obtained when the groups received the check 22 per cent mixture. The average production was 42.5 lbs. when the Cornell test mixture was fed; 42.4 lbs. of 4 per cent F.C.M. were produced when the ground whole grains mixture and the by-products mixture were fed. The greatest average difference in production on these four mixtures was only 0.5 lb. of milk per day. This difference was not statistically significant. The variation in production on the different concentrate mixtures was considerably less than the differences among the experimental groups. Also,

TABLE 3

Summary of the daily production of 4 per cent fat-corrected milk

| Group | Av. production of 4 per cent F.C.M. per day | | | | |
	Cornell test mixture	Check 22% mixture	Ground whole grains mixture	By-products mixture	Av. production
	(lbs.)	*(lbs.)*	*(lbs.)*	*(lbs.)*	*(lbs.)*
I	47.2 (period 1)	43.6 (period 2)	40.5 (period 3)	36.0 (period 4)	41.8
II	35.2 (period 4)	46.1 (period 1)	43.7 (period 2)	39.2 (period 3)	41.1
III	43.2 (period 3)	40.1 (period 4)	47.3 (period 1)	46.5 (period 2)	44.3
IV	44.2 (period 2)	41.7 (period 3)	38.1 (period 4)	47.8 (period 1)	43.0
Av. production	42.5	42.9	42.4	42.4	42.6

there was less difference in production on the Cornell test mixture and the ground whole grains mixture than was shown in the double reversal trial in experiment A.

Feed consumption was practically the same for all groups. Since the concentrate portion of the ration was fed according to production, the higher producing groups received slightly more concentrates. The change in body weight was a general trend toward a slight gain in body weight throughout the experimental period.

DISCUSSION

This series of experiments has several practical implications. In periods when some of the standard ingredients in feed mixtures are difficult to obtain, the results of these experiments indicate that substitutions may be made on a rather wide basis without altering the feeding value or palatability so long as the total protein and total digestible nutrients remain fairly constant. If it is economical to do so, the results indicate that by-product

feeds of the vegetable oil-producing and milling industries may replace farm-grown grains entirely, and vice versa, to a certain extent, without noticeably affecting the nutritive value of the mixture. In fact, such a procedure is rather common practice among feed mixers in producing economical feeds from ingredients that change in their price relationships to one another from time to time. Although not a commonly recommended feeding practice, abrupt changes in the concentrate mixture may be effected without producing harmful results.

SUMMARY

A series of three experiments comparing the feeding value of different concentrate mixtures indicated that there was little or no difference in the palatability of concentrate mixtures that differed widely in the ingredients used. A mixture containing 76 per cent of farm-grown grains was equal in promoting milk production and in palatability to a standard concentrate mixture throughout a continuous study of 26 weeks' duration. Similar results were obtained in a double reversal trial.

Another experiment involving four concentrate mixtures containing widely different ingredients showed there was little difference in feeding value among the four mixtures. Abrupt changes from one mixture to another had no unfavorable effect on feed consumption. All mixtures proved equally palatable. Body weight essentially was unaffected by the different concentrate mixtures.

REFERENCES

(1) BRANTON, C. C., RUSOFF, L. L., AND SEATH, D. M. Simple vs. Complex Dairy Rations. Dairy Res. Digest (La. Sta.) 1(3): 1. 1943.

(2) DAWSON, J R., WATT, A. L., MCINTYRE, C. W., LEIGHTON, R. E., AND GRAVES, R. R. Single Grains and Grain Mixtures as Supplements to Alfalfa Hay and Silage for Milk Production. U. S. Dept. Agr. Cir. 696. 1944.

(3) MONROE, C. F., AND KRAUSS, W. E. Simple vs. Complex Rations for Dairy Cattle. Jour. Dairy Sci., 25: 673–674. 1942.

(4) MONROE, C. F., AND KRAUSS, W. E. Single vs. Complex Grain Mixtures in Dairy Rations. I. Winter Feeding of Milking Cows. Ohio Agr. Expt. Sta. Bimo. Bul. 238: 19–24. 1946.

(5) MONROE, C. F., AND KRAUSS, W. E. Simple vs. Complex Grain Mixtures in Dairy Rations. II. Summer Feeding to Milk Cows on Pasture. Ohio Agr. Expt. Sta. Bimo. Bul. 239: 31–35. 1946.

(6) MONROE, C. F., AND LIVESEY, WALTER. Simple vs. Complex Grain Mixtures in Dairy Rations. V. Continuous Feeding Trial with Milking Cows. Ohio Agr. Expt. Sta. Bimo. Bul. 242: 123–130. 1946.

(7) MORRISON, F. B., BRATTON, R. W., AND SALISBURY, G. W. Influence of Quality of Protein in the Concentrate Mixture on Production of Dairy Cows Fed Mixed Hay and Corn Silage. N. Y. (Cornell) Agr. Expt. Sta. Ann. Rpt., 1942: 103–104. 1942.

(8) WILLIAMS, N. K., CANNON, C. Y., AND ESPE, D. L. Production of Dairy Cows When Fed Only Silage and Cracked Soybeans. Jour. Dairy Sci., 19: 459. 1936.

THE HEAT RESISTANCE OF LACTOBACILLI FOUND IN AMERICAN CHEDDAR CHEESE[1]

W. L. SLATTER[2] AND H. O. HALVORSON[3]

University of Minnesota, St. Paul

The heat treatment of milk for cheesemaking is not a new development. However, pasteurization as ordinarily defined has not been used extensively in the cheese industry, the temperature and time of exposure often being less than the minimum required by public health officials. A number of states have passed laws requiring that cheese either be made from pasteurized milk as defined by public health ordinances or be aged a specified length of time. Such laws affect not only the cheese made in these states but also all the cheese produced in other states for sale in the areas concerned.

Experience has shown that Cheddar cheese made from pasteurized milk rarely develops the full, characteristic flavor ordinarily found in good quality raw milk cheese, even after an extended ripening period (11, 19, 20). This may be due to the destruction of enzymes, destruction of microorganisms, or to a chemical change in the milk as a result of pasteurization. Bacteriological studies have shown that lactobacilli often grow extensively in Cheddar cheese and frequently are present in tremendous numbers after ripening for several weeks (1, 15, 16, 17, 21, 22, 23). This suggests that these organisms may be important in the cheese-ripening process. The reason pasteurized milk cheese ripens more slowly may be that many of these organisms are destroyed in the pasteurizing process. The object of this investigation was to study the heat resistance of lactobacilli found in Cheddar cheese.

REVIEW OF LITERATURE

The standard of comparison of heat tolerance of different species of bacteria originally was the thermal death point, *i.e.*, the lowest temperature at which a suspension of bacteria could be killed in 10 minutes. This method cannot give comparable results unless conditions such as age of culture, approximate number of cells, pH of suspension, dimensions of test tubes, and thickness of glass in the test tubes are standardized.

The idea has since gained acceptance that (a) there is no critical lethal temperature, (b) any temperature high enough to have an unfavorable effect upon the growth and stamina of the bacteria is lethal and (c) bac-

231

teria will be destroyed if they are subjected to an unfavorable temperature long enough. Research workers in the canning industry have found it more suitable for their purposes to keep the temperature constant and to vary the time. The thermal death time is considered to be the shortest time necessary to kill all bacteria in a given suspension at a given temperature.

Since the basic work of Kröng and Paul (19) in 1897, it has been recognized that the mortality rate of bacteria exposed to unfavorable conditions follows a generally regular and consistent course. Under a wide variety of conditions, this course is such that a straight line is obtained when the log of the number of bacteria surviving at a given moment is plotted against the time elapsed since the beginning of the experiment.

The order of death of spore-forming bacteria has been found to be logarithmic by Chick (10), Bigelow (6), Weiss (27), Esty and Meyer (11), and Watkins and Winslow (26).

Bigelow and Esty (8) were perhaps the first to consider some of the factors now recognized as very important, and they proposed a standard technic for determining the resistance of organisms to heat. They proposed an accurately controlled oil bath and special thermal-death-time tubes of soft glass 250 mm. long, 7 mm. inside diameter, and with a wall thickness of 1 mm. The spores were suspended in juices expressed from various canned foods which had received one heat treatment. When a juice had been inoculated with a spore suspension, it was introduced into the thermal-death-time tube, which then was sealed and placed in an oil bath for the heat treatment. Fifteen seconds were allowed for the tubes to come to bath temperature before time was counted. This method was designated as the "single tube" method in contrast to one proposed later by Esty and Williams (12). One difficulty with the single tube method was described as "skips", *i.e.*, destruction of organisms at shorter times when longer heating would leave viable cultures.

In 1924 Esty and Williams (12) introduced a "multiple tube" method to reduce the number of skips. Instead of heating one tube for a given time, 25 to 30 tubes were heated, each containing a portion of the same suspension, and all were heated alike for at least four different time intervals. These intervals were selected to cover the entire range of heat resistance based on percentage survival.

Bigelow (6), using the thermal-death-time data reported by Bigelow and Esty (8), plotted the results on semi-logarithmic paper instead of coordinate paper. The curves were drawn so that they passed between the time intervals representing the last positive and the first negative thermal death tubes in the greatest number of pairs of observation points with each organism. Such curves were straight lines or nearly so, and from them was secured the thermal death time of the organism between the temperatures which were used to construct the curve. Bigelow's work was done with spore-forming,

thermophilic organisms. Since no data were available for non-spore-forming bacteria, he determined the heat resistance of four such organisms (*Bacterium alkaligenes*, *Bacterium coli*, *Bacterium aerogenes*, and *Bacterium proteus*) at temperatures of 40° C. (104° F.) to 65° C. (149° F.) at 5°-intervals. He did not find as consistent results with these organisms as he did with the spore-forming, thermophilic bacteria. However, he concluded that the thermal-death-time curves for these organisms were logarithmic.

Since Bigelow's suggestion that death of non-spore-forming bacteria was logarithmic, Watkins and Winslow (26), Beamer and Tanner (5), and Baker and McClung (2) have published confirmatory results.

Since bacteria follow a more or less uniform logarithmic order of death, death rates can be computed and conclusions drawn from them. Bigelow, Bohart, Richardson, and Ball (7) described the "general method" for making calculations of processing times for canned foods. One of the requirements was that the thermal death time of the organisms being destroyed by the process must be known at all temperatures attained during the process. This knowledge was obtained by determining the thermal death times at several temperatures in the processing range. The data so obtained were plotted, using a logarithmic time scale and a linear temperature scale. The resulting points were connected by a smooth curve. From this curve, thermal death times were found for all temperatures obtained during the heating process. It was not necessary to know the mathematical formula relating the thermal death time to the temperature.

Ball (3, 4) suggested some improvements for the "general method" of process calculations used in the study of temperatures required in the canning of vegetables. He did not use death rates or temperature coefficients but the factors F and z. The value F_1 was the thermal death time of the bacterial species at 121° C. (250° F.), while the value F represented the thermal death time at any other temperature. The letter z referred to the temperature increase in degrees Fahrenheit necessary to reduce the death time one-tenth. The value of z indicated the slope of the straight line obtained by plotting the logarithms of death time against temperature. The value F gave one point on the curve. Therefore, F and z were sufficient to characterize the thermal resistance of the bacterial spores at any temperature.

When making thermal-death-time tests involving relatively short times (less than 10 minutes), the heat penetration lag, or the time for the thermal-death-time tube and its contents to come up to the temperature of the constant-temperature bath, may make up an appreciable percentage of the total death time (24). Many investigators apparently have ignored this fact. Bigelow and Esty (8), Weiss (27), Esty and Meyer (11), and Esty and Williams (12) used a series of glass tubes containing suspensions of heat-resistant bacteria which were heated in oil baths. Not more than 15 seconds

were allowed for lag in heat penetration. Townshend (25) measured the heat resistance of spore-forming anaerobes, using a water bath. He allowed a lag correction of 1 minute in the heating times. Sognefest and Benjamin (24) measured the heating lag in Pyrex thermal-death-time tubes by means of a thermocouple when various media such as water, sugar solutions, and vegetable juices were placed in the tubes. The correction factor for water figured for an organism with a z value of 18 was 0.85 minute when heated in a water bath. Gross (14) used Kimble brand no. 45050 chemical test tubes in thermal-death-time studies of a staphylococcus in a meat-juice medium heated in an oil bath. The lag on these tubes was measured by means of a thermocouple and found to be approximately 3 minutes when heated to 140–160° F.

EXPERIMENTAL METHODS

Samples of cheese were obtained, using sterile triers, and the samples transferred to sterile sample jars. An 11-g. sample of each cheese was transferred to a sterile mortar and ground to a homogeneous suspension with the aid of a pestle, a small amount of sterile sand, and the addition of part of the water from a 99-ml. sterile water dilution blank, the mixture representing a 1 to 10 dilution of the cheese. From this dilution, other desired dilutions were prepared. The various dilutions then were plated, using Difco tomato juice agar containing 400 ml. tomato juice, 10 g. Bacto peptone, 10 g. Bacto peptonized milk, and 11 g. Bacto agar per liter. The plates were incubated at room temperature (21° C.) for 10 days. Twenty-five contiguous colonies were picked from one plate in each set and inoculated into tubes of sterile litmus milk. After 10 to 14 days incubation at room temperature, the appearance of each litmus milk culture and the morphological characteristics of the organism were recorded. The cultures were stained with the gram stain using the Burke (9) modification. Non-spore-forming, grampositive rods that reduced and coagulated litmus milk in 10 to 14 days at room temperature were selected as lactobacilli. The length and width of cells and the speed of growth in litmus milk were considered in the selection of cultures for the heat-resistance studies. Not more than two cultures were selected from any one cheese. The cultures selected were streaked on tomato juice agar, covered with another layer of agar, and incubated for 10 days at room temperature. Colonies then were picked into litmus milk and incubated again for 10 days at room temperature. The morphology of these cultures was observed, using the gram stain, and one culture was selected for the heat-resistance studies. The cultures selected were inoculated into glucose, galactose, lactose, fructose, maltose, mannite, mannose, salicin and inulin broths.

The cultures were numbered as follows: the first number refers to the number of the cheese in which the organism was found, the second to the

number of the colony picked from the plate used in the isolation, and the
third to the colony picked when the cultures were streaked on tomato juice
agar. A record was kept of morphology, colony, and fermentation character-
istics so that it was possible to get the history of a culture whenever necessary.

The method employed for the heat-resistance studies was similar to that
used by Bigelow and Esty (8), with litmus milk made from fresh skim milk.
One drop (from a 2.2-ml. pipette) of each lactobacillus culture was trans-
ferred into a 10-ml. sterile, skim milk dilution blank. The dilution blank
was shaken vigorously for 1 minute. One milliliter of this dilution was
added to each 100 ml. of sterile litmus milk to be inoculated for the heat
treatment. The inoculated litmus milk was allowed to remain over night at
5° C. and then transferred to sterile, Kimble brand no. 45050 (10 × 75 mm.)
chemical test tubes and the tubes sealed. The number of bacteria in the
inoculum was determined by plating various dilutions of the litmus milk on
tomato juice agar.

The sealed tubes were submerged in a De Khotinsky constant-temperature
water bath with a maximum temperature variation of ± 0.2° F. The tubes
were exposed in the constant-temperature bath for varying periods of time,
the time intervals being measured with a stop watch. When the tubes were
taken from the water bath, they were immersed at once in water at 60–65° F.
to cool. The tubes then were allowed to incubate 4 weeks at 30° C., after
which all tubes were observed for growth. The first negative tubes in a
series frequently were plated or observed under a microscope to be sure no
viable organisms were present. Single tubes were heated at 5-minute in-
tervals to get a general picture of the heat resistance of the lactobacillus
cultures. To determine the z values, ten tubes were removed at from 1- to
5-minute intervals at each of four different temperatures.

In order to determine the heat lag on the Kimble tubes used in the heat-
resistance studies, a skim milk thermometer was made by attaching a 29-inch
capillary tube to one of the Kimble tubes. A vacuum was pulled on the test
tube with the capillary tube attached, and the test tube filled with skim
milk containing a few drops of formaldehyde. It then was attached to a
meter stick and calibrated against a mercury thermometer by holding in a
water bath at different temperatures. To get the heat lag, this milk ther-
mometer was placed in a constant-temperature bath at various tempera-
tures and the meter stick reading recorded every 15 seconds. To get the
cooling lag, the milk thermometer was transferred directly from the con-
stant-temperature bath to water at 65° F. and the readings recorded every
15 seconds until it reached 65° F. The time of heating and cooling was
converted to an equivalent time at the temperature of the constant-tem-
perature bath by a method of graphic integration.

The temperature lag correction, when heating a skim milk medium in
water to 135°, 145°, and 155° F., using z values from 8 to 12, was found

W. L. SLATTER AND H. O. HALVORSON

TABLE 1

Thermal death times at 145° F. of cultures from raw milk cheese

	Period of exposure in minutes									
	< 5	10	15	20	25	30	35	50	60	Over 60
					Culture nos.					
	3–8–5	11–9–5	6–15–5	7–14–6	10–1–8	15–5–1	8–6–3	8–16–3	17–6–3	13–20–6
	4–17–4	16–14–1	11–14–5	23–1–3	10–2–8	3–13–4		20–8–1	19–1–6	14–4–4
	7–13–6	24–14–4	15–27–1	25–15–6	12–27–6	4–27–6				19–27–6
	9–7–2	24–20–5	16–11–3		13–26–6	6–25–7				
	9–17–1		17–21–1		14–5–1					
	12–9–2		21–8–1		20–6–6					
	18–1–5		25–4–6		22–19–6					
	18–19–6		32–1–1		2–14–6					
	21–11–6		32–6–1							
	22–7–4									
	23–19–1									
	2–1–7									

to be approximately 1 minute. One minute was subtracted from the time that the tubes were exposed to the constant temperature to get the lethal time of exposure in the constant-temperature bath.

Thirty-two cheeses made in various parts of Wisconsin and Minnesota were the source of the organisms used in these experiments. Twenty-four of the cheeses were made from raw milk. Two cheeses were made from milk pasteurized at 145° F. for 30 minutes, while six were made from milk flash-heated, the temperature fluctuating from 155° to 170° F. The age of the cheese varied from 1 week to 2 years.

RESULTS

Eight hundred colonies were picked from the 32 cheeses; 448 colonies were of organisms of the rod type. Of the 25 colonies picked from a plate from each cheese, the types varied from all rods to all cocci. Very few rod types were found in the 2-year-old cheese. In the other cheese, many had a very high proportion of rods. When inoculated into litmus milk, 439 cultures were found to be acid coagulating, 345 acid non-coagulating, 12 formed a yellow sediment at the bottom of the litmus milk tubes, and 6 cultures digested the milk solids. Sixty cultures were selected for further study on the basis of morphology, growth in litmus milk, and the source of the culture (raw or pasteurized milk cheese).

A summary of the thermal death times at 145° F. of lactobacilli from raw milk cheese is given in table 1. Twelve cultures could be killed in less

TABLE 2

Thermal death times at 136° F. of cultures from raw milk cheese killed in less than 5 minutes at 145° F.

Period of exposure at 136° F.	Thermal death time of culture no.										
	18-1-5	12-9-2	9-17-1	9-7-2	21-11-6	18-19-6	23-19-1	22-7-4	3-8-5	4-17-4	7-13-6
Minutes											
0	+	+	+	+	+	+	+	+	+	+	+
5	+	−	+	+	+	+	+	+	+	+	+
10	+	−	+	+	−	+	−	+	−	+	+
15	+	−	+	+	−	+	−	−	−	+	−.
20	+	−	−	−	−	+	−	−	−	−	−
25	+	−	−	−	−	−	−	−	−	−	−
30	+	−	−	−	−	−	−	−	−	−	−
35	−	−	−	−	−	−	−	−	−	−	−
40	−	−	−	−	−	−	−	−	−	−	−
45	−	−	−	−	−	−	−	−	−	−	−
Plate count per ml. of litmus milk culture (thousands)	1	< 1	11	72	130	< 1	160	6	4	< 1	3

+ Indicates growth.
− Indicates no growth.

TABLE 3

Thermal death times at 154° F. of cultures from raw milk cheese not killed in 60 minutes at 145° F.

Period of exposure at 154° F.	Thermal death time of culture no.		
	13–20–6	14–4–4	19–27–6
Minutes			
0	+	+	+
5	+	+	+
10	−	−	−
15	−	−	−
20	−	−	−
Plate count per ml. of litmus milk culture	124,000	58,000	120,000

+ Indicates growth.
− Indicates no growth.

than 5 minutes, four in 10 minutes, nine in 15 minutes, three in 20 minutes, eight in 25 minutes, four in 30 minutes, one in 35 minutes, two in 50 minutes, and two in 60 minutes, while three cultures could not be killed in 60 minutes. Approximately 83 per cent of the cultures could be killed in 30 minutes or less at 145° F.

The thermal death times at 136° F. of cultures from raw milk cheese, which could be killed in less than 5 minutes at 145° F., are given in table 2. The thermal death time varied from less than 5 minutes to 35 minutes, with nine of the eleven cultures being killed in from 10 to 20 minutes.

The thermal death times at 154° F. of cultures from raw milk cheese not killed in 60 minutes at 145° F. are given in table 3. All three of these cultures could be killed in 10 minutes at 154° F.

TABLE 4

Thermal death times at 154° F. of cultures from pasteurized milk cheese

Period of exposure at 154° F.	Thermal death time of culture no.									
	5–6–6	5–8–9	26–2–1	26–6–1	27–2–1	27–19–1	28–2–1	28–16–2	30–1–1	30–20–1
Minutes										
0	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	−	+	+	+
15	+	+	+	−	+	+	−	+	+	+
20	+	+	+	−	−	+	−	−	−	+
25	+	−	−	−	−	−	−	−	−	−
30	−	−	−	−	−	−	−	−	−	−
35	−	−	−	−	−	−	−	−	−	−
40	−	−	−	−	−	−	−	−	−	−
Plate count per ml. of litmus milk culture (thousands)	100	130	235	17,000	100	3.800	51	122	73	3,000

+ Indicates growth.
− Indicates no growth.
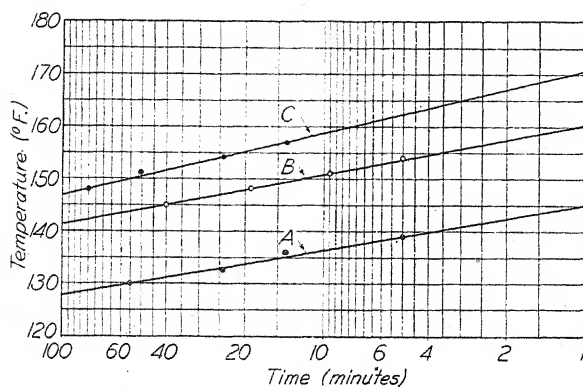
FIG. 1. Thermal-death-time curves: Curve A, Culture no. 3–8–1, z value = 8.5. Curve B, Culture no. 8–16–3, z value = 9.5. Curve C, Culture no. 5–6–6, z value = 12.

The thermal death times at 154° F. of cultures from pasteurized milk cheese are given in table 4. The thermal death times varied from 10 to 30 minutes. The colonies of this group of organisms on tomato juice agar commonly were very small, approaching pin-point size.

The fermentation characteristics of all cultures isolated were studied and found to have little or no correlation with their heat resistance.

The heat resistance of the organisms was the only factor considered in selecting the cultures for the thermal-death-time curve studies. Two cultures (3–8–1 and 11–9–5) having a low heat resistance, three (8–16–3, 6–25–7, and 19–1–6) having a medium heat resistance, and two (5–6–6 and 30–20–1) having the maximum heat resistance were selected.

Culture no. 3–8–1 could be killed within 5 minutes at 139°, 14 minutes at 136°, 24 minutes at 133°, and 55 minutes at 130° F. When these data
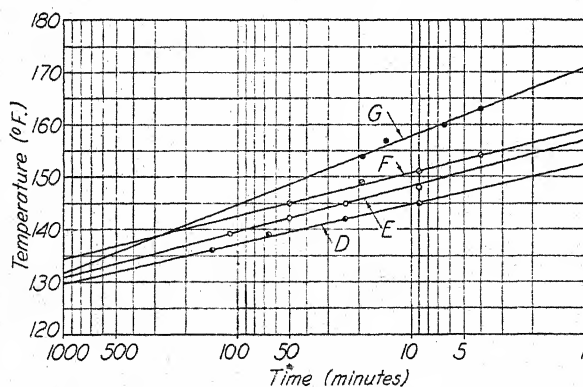
FIG. 2. Thermal-death-time curves: Curve D, Culture no. 11–9–5, z value = 8. Curve E, Culture no. 6–25–7, z value = 8. Curve F, Culture no. 19–1–6, z value = 8.5. Curve G, Culture no. 30–20–1, z value = 13.

were plotted on semi-logarithmic paper, they gave curve A shown in figure 1. The z value (slope of the curve) for this culture was approximately 8.5.

Culture no. 8–16–3 could be killed in 5 minutes at 154°, 9.5 minutes at 151°, 19 minutes at 148°, and 40 minutes at 145° F. When these data were plotted, they gave curve B (fig. 1). The z value was approximately 9.5.

Culture no. 5–6–6 could be killed in 14 minutes at 157°, in 24 minutes at 154°, in 50 minutes at 151°, and in 80 minutes at 148° F. These data gave thermal-death-time curve C (fig. 1). The z value was 12.

Culture no. 11–9–5 could be killed within 9 minutes at 145°, 24 minutes at 142°, 65 minutes at 139°, and 140 minutes at 136° F. These data gave curve D (fig. 2). The z value was 8.

Culture no. 6–25–7 could be killed within 9 minutes at 148°, in 24 minutes at 145°, in 50 minutes at 142°, and 110 minutes at 139° F. These data gave curve E (fig. 2), indicating this culture had a z value of approximately 8.

Culture no. 19–1–6 could be killed in 4 minutes at 154°, 9 minutes at 151°, 19 minutes at 149°, and 50 minutes at 145° F. These data gave curve F (fig. 2). The z value was 8.5.

Culture no. 30–20–1 could be killed in 4 minutes at 163°, 6.5 minutes at 160°, 14 minutes at 157°, and 19 minutes at 154° F. These data gave curve G (fig. 2), indicating a z value of approximately 13.

The lactobacilli that could be killed in 30 minutes or less at 145° F. had z values varying from 8 to 8.5, while the organisms that could be killed in from 30 to 60 minutes at 145° F. had z values from 8.5 to 9.5. The organisms having a thermal death time of over 60 minutes at 145° F. had z values varying from 12 to 13.

DISCUSSION

The heat resistance of lactobacilli found in Cheddar cheese varied within rather wide limits. Some of this variation undoubtedly was due to the differences in the numbers of organisms in the cultures used for the heat-resistance trials. However, this variation was not great when the thermal death time was less than 30 minutes at any temperature. It usually was possible to take different cultures of the same strain of lactobacilli and have the thermal death time on successive trial runs check within 5 minutes. When the thermal death time was over 30 minutes, a much greater variation frequently was observed. Since the number of lactobacilli in a normal raw milk supply is comparatively low (1), the heat resistance data reported in this study may suggest a greater heat resistance for some organisms than would actually be the case in a raw milk supply. This observation seems justified because of the large numbers of organisms present in some of the cultures used for these heat-resistance trials.

In this study the percentage of lactobacilli destroyed by pasteurization exposure was considered more important than the variability of the heat resistance. Approximately 83 per cent of the lactobacilli found in Cheddar cheese made from raw milk could be killed in 30 minutes or less at 145° F. The lactobacilli found in Cheddar cheese made from pasteurized milk were much more heat resistant, having a thermal death time of from 10 to 35 minutes at 154° F. This might explain why Evans, Hastings, and Hart (13) found only one-tenth as many *Lactobacillus casei* in pasteurized milk cheese as in raw milk cheese made from the same milk up to the forty-second day of ripening. The destruction of lactobacilli by pasteurization may be an important reason why cheese made from pasteurized milk ripens more slowly than cheese made from raw milk. This suggests the possibility that the ripening of cheese made from pasteurized milk may be accelerated either by adding the proper lactobacillus cultures or by ripening the cheese at a higher temperature in order to supply a more favorable growth temperature for the reduced numbers of lactobacilli that survive pasteurization.

The fermentation characteristics of the lactobacilli found in Cheddar cheese had very little correlation with heat resistance. When the organisms were grouped according to their heat resistance, the more organisms in any one group the fewer the fermentation characteristics they had in common.

The temperature lag on the Kimble brand test tubes used was approximately 1 minute in a water medium. This checks closely with the correction determined by Gross (14) using the same tubes and by Sognefest and Benjamin (24) using similar tubes.

The z values for the lactobacilli varied from eight to thirteen, with the most heat-resistant organisms having the highest z values. A thermal-death-time curve with a slope of 8 passing through a point at 145° F. for 30 minutes shows that the lactobacilli which could be killed in 30 minutes or less at 145° F. also could be killed in 27 seconds at 160° F. or 7 seconds at 165° F. This would include 83 per cent of the lactobacilli found in Cheddar cheese made from raw milk, as milk for cheesemaking commonly is pasteurized at 165° F. for 15 seconds. Most ordinances require a minimum time and temperature exposure of 143° F. for 30 minutes or 160° F. for 15 seconds for public health reasons. These heat exposures would destroy approximately 52 per cent of the bactobacilli found in raw milk cheese. Using the minimum exposures would permit a significant increase in the number of lactobacilli surviving pasteurization.

Some skips were encountered, especially where the thermal death time was over 1 hour. Few skips were encountered when the thermal death time was less than 30 minutes at any temperature.

SUMMARY AND CONCLUSIONS

1. The heat resistance of 60 lactobacillus cultures found in Cheddar

cheese made from raw milk was studied and found to vary within wide limits.

2. The majority of lactobacilli found in Cheddar cheese made from raw milk can be killed by pasteurizing at 143° F. for 30 minutes or 160° F. for 15 seconds.

3. The fermentation characteristics of the lactobacilli found in Cheddar cheese had little correlation with their heat resistance.

4. The *z* values vary in a general way with the heat resistance of the organisms, the most heat-resistant lactobacilli having the highest *z* values.

5. The destruction of lactobacilli by pasteurization suggests the possibility of accelerating the ripening of Cheddar cheese made from pasteurized milk either by adding the proper lactobacillus culture to the milk or by raising the ripening temperature of the cheese to supply a more favorable growing temperature for the reduced numbers of lactobacilli that survive pasteurization.

REFERENCES

(1) ALLEN, L. A., AND KNOWLES, N. R.   Studies in the Ripening of Cheddar Cheese. IV. The Bacterial Flora of Cheese Made from Milk of Very Low Bacterial Count. V. The Influence of the Rennet Flora on the Flora of the Cheese. VI. The Influence of Starter on the Ripening Process. Jour. Dairy Res., **5**: 185–195. 1934.

(2) BAKER, E. E., AND McCLUNG, L. S.   Determination of the Heat Resistance of Non-spore-forming Bacteria. Food Res., **4**: 21–29. 1939.

(3) BALL, C. O.   Thermal Process Time for Canned Food. Natl. Res. Council Bul. 37. 1923.

(4) BALL, C. O.   Mathematical Solution of Problems in Thermal Processing of Canned Food. University of Calif. Pub. in Pub. Health 1(2): 15–245. 1928.

(5) BEAMER, P. R., AND TANNER, F. W.   Resistance of Non-spore-forming Bacteria to Heat. Zentbl. f. Bakt. Abt. II, **100**: 81–98. 1939.

(6) BIGELOW, W. D.   The Logarithmic Nature of Thermal-death-time Curves. Jour. Infect. Dis., **29**: 528–536. 1921.

(7) BIGELOW, W. D., BOHART, G. S., RICHARDSON, A. C., AND BALL, C. O.   Heat Penetration in Processing Canned Foods. Natl. Canners Assoc. Bul. 16L. 1920.

(8) BIGELOW, W. D., AND ESTY, J. R.   The Thermal Death in Relation to Time of Typical Thermophilic Organisms. Jour. Infect. Dis., **27**: 602–617. 1920.

(9) BURKE, VICTOR.   Notes on the Gram Stain with Description of a New Method. Jour. Bact., **7**: 159–182. 1922.

(10) CHICK, H.   The Process of Disinfection by Chemical Agencies and Hot Water. Jour. Hyg., **10**: 237–286. 1910.

(11) ESTY, J. R., and MEYER, K. F.   The Heat Resistance of the Spores of *B. botulinus* and Allied Anaerobes. Jour. Infect. Dis., **31**: 650–663. 1922.

(12) ESTY, J. R., AND WILLIAMS, C. C.   Heat Resistance. I. A New Method for the Determination of Heat Resistance of Bacterial Spores. Jour. Infect. Dis., **34**: 516–528. 1924.

(13) EVANS, ALICE, HASTINGS, E. G., AND HART, E. B.   Bacteria Concerned in the Production of the Characteristic Flavor in Cheese of the Cheddar Type. Jour. Agr. Res., **2**: 167–192. 1914.

(14) GROSS, C. E.   John Morrell & Company, Ottumwa, Iowa.   Personal Communication. 1945.

(15) HART, E. B., HASTINGS, E. G., FLINT, E. M., AND EVANS, ALICE C.   Relation of the Action of Certain Bacteria to the Ripening of Cheese of the Cheddar Type. Jour. Agr. Res., 2: 193–216. 1914.

(16) HASTINGS, E. G., EVANS, ALICE C., AND HART, E. B.   Studies on the Factors Concerned in the Ripening of Cheddar Cheese. Wis. Agr. Expt. Sta. Res. Bul. 25. 1912.

(17) HUCKER, G. J.   The Types of Bacteria Found in Commercial Cheddar Cheese. N. Y. (Geneva) Agr. Expt. Sta. Tech. Bul. 90. 1922.

(18) KELLY, C. D.   Pasteurization of Milk for Cheesemaking. N. Y. (Geneva) Agr. Expt. Sta. Cir. 175. 1937.

(19) KRÖNG, B., AND PAUL, T.   Die chemische Grundlagen der Lehre von der Giftwirkung und Desinfektion. Atschn. f. Hyg., 25: 1–112. 1897.

(20) LANE, C. B., AND HAMMER, B. W.   Studies on the Bacteriology of Cheese. I. Effect of Pasteurizing Milk on the Nitrogenous Decomposition in Cheddar Cheese. Iowa Agr. Expt. Sta. Res. Bul. 183. 1935.

(21) LANE, C. B., AND HAMMER, B. W.   Bacteriology of Cheese. II. Effect of *Lactobacillus casei* on the Nitrogenous Decomposition and Flavor Development in Cheddar Cheese Made from Pasteurized Milk. Iowa Agr. Expt. Sta. Res. Bul. 190. 1935.

(22) SHERWOOD, I. R.   The Bacterial Flora of New Zealand Cheddar Cheese. Jour. Dairy Res., 10: 426–448. 1939.

(23) SHERWOOD, I. R.   Lactic Acid Bacteria in Relation to Cheese Flavor. II. Observations on the Inoculations of the Milk Employed in Cheese Manufacture with Lactobacilli. Jour. Dairy Res., 10: 449–454. 1939.

(24) SOGNEFEST, P., AND BENJAMIN, H. A.   Heat Lag in Thermal-death-time Cans and Tubes. Food Res., 9: 234–243. 1944.

(25) TOWNSHEND, C. T.   Spore-forming Anaerobes Causing Spoilage in Canned Foods. Food Res., 4: 231–237. 1939.

(26) WATKINS, J. H., AND WINSLOW, C.-E. A.   Factors Determining the Rate of Mortality of Bacteria Exposed to Alkalinity and Heat. Jour. Bact., 24: 243–265. 1932.

(27) WEISS, H.   The Heat Resistance of Spores with Special Reference to the Spores of *B. botulinus*. Jour. Infect. Dis., 28: 70–92. 1921.

THE STABILIZATION OF CAROTENE IN DEHYDRATED LEGUMES (ALFALFA) AND CEREAL GRASSES[1,2]

A. W. HALVERSON AND E. B. HART

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

In an earlier study on the stabilization of carotene (2) in dehydrated feeds and foods, consideration of various physical and chemical treatments was made.

Additional heat treatments were effective in reducing the loss of carotene in dehydrated oats from 70–80 per cent to 30–50 per cent in 6 months storage. The addition of 0.9 per cent of diphenylamine to dehydrated oats decreased the loss of carotene from 77 to 41 per cent in 6 months. Experiments with a number of chemical agents which changed the reaction of the material or of substances which could act as reducing agents or acceptors of oxygen were ineffective. Pelleting and coating with flexo wax reduced the loss from 74 to 45 per cent in 6 months. Attempts to remove the oxygen from the pellets before coating by washing with nitrogen also helped materially in decreasing the carotene loss.

Further studies (3) showed that autoclaving dehydrated oats or alfalfa at 15 lbs. pressure for 1 hour and then pressing into large pellets (3×4 inches) and dipping in flexo wax, reduced the loss in dehydrated alfalfa to 28 per cent in 3 months and to 0 per cent in the case of dehydrated oats.

EXPERIMENTAL

Additional studies now have been made on the influence of time of autoclaving on the stabilization of carotene, as well as pelleting and nitrogen washing before pelleting and waxing. In table 1 the data show the effect of autoclaving for 1 hour in the absence of oxygen. In this process the chlorophyll is destroyed and the material is of dark brownish color. Carotene determinations were made by the method outlined previously (1). These data show that mere autoclaving to assure complete destruction of the "lipoxidase", which can bring about the destruction of carotene, had no significant effect in checking carotene losses. Only when oxygen is excluded, either in part or, preferably, completely, is preservation of the carotene brought to a comparatively high percentage. Washing with nitrogen before pressing was quite effective in further reducing the loss. However, after washing in nitrogen, the material was exposed to air in the process of pellet making.

In table 2 are the records of the effect of time of autoclaving on the carotene conservation. These data show that autoclaving for 5 minutes gave results similar to those secured with 60 minutes of autoclaving; carotene was well preserved in the material that had not been autoclaved at all but protected from oxygen through pelleting and waxing, or, even better, by washing with nitrogen and then pelleting in air and waxing. These data are in harmony with earlier observations by other investigators. The carotene can be preserved in plant tissues where the dehydrated material is stored in an inert gas such as nitrogen (1). Mere destruction of the carotene oxidizing

TABLE 1

Effect of autoclaving for 1 hour on the stability of carotene in dehydrated cereal grasses and alfalfa kept at 22–25° C.

(All samples pressed[1] into large pellets 3 × 4 in.)

Material and treatment	Carotene content, μg./g. (air-dry basis)			% loss 6 mos.
	Initial	3 mos.	6 mos.	
Dehydrated oats				
No treatment	450	155	116	74.2
Autoclaved	423	171	74	82.5
Auto. + flexo waxed	423	379	335	20.8
Auto. + flexo waxed + N₂ washing	423	417	368	13.0
Dehydrated sudan grass				
No treatment	299	96	83	72.2
Autoclaved	323	175	136	57.9
Auto. + flexo waxed	323	191	171	47.0
Auto. + flexo waxed + N₂ washing	323	260	256	20.7
Dehydrated alfalfa				
No treatment	222	88	72	67.6
Autoclaved	235	117	75	68.1
Auto. + flexo waxed	235	154	151	35.8
Auto. + flexo waxed + N₂ washing	235	160	173	26.5
Alfalfa dried at 50° C.				
No treatment	236	154	153	35.2
Autoclaved before drying	334	190	129	61.4
Auto. + flexo waxed	334	280	282	15.5
Auto. + flexo waxed + N₂ washing	334	262	313	6.3

[1] Carver press: 1,500 lbs. per sq. in.

enzyme, lipoxidase, will not lead to carotene preservation. Oxygen must be excluded or auto-oxidation of the carotene will proceed.

In further experiments small tubular cellophane casings were used. Into these casings the commercially dehydrated oat material was lightly pressed and the ends merely twisted together and tied with a string. Various treatments were given the material before and after placing in the casings. All of the samples were mixed with water to a content of 15 per cent, giving a total initial moisture content of about 22 per cent. After mixing with the water some of the samples were dried at 95° C. for varying lengths of time. In table 3 the treatments of the materials and the results secured are given.

TABLE 2

Effect of time of autoclaving on preservation of carotene in dehydrated cereal grasses (oats) and alfalfa kept at 22–25° C.

(Pressed into large pellets 3 × 4 in.)

Material and treatment	Carotene content, µg./g. (air-dry basis)		% loss 3 mos.
	Initial	3 mos.	
Dehydrated oats			
Unheated	380	212	44.0
Unheated + flexo wax	380	280	26.0
Unheated + flexo wax + N_2 washing	380	390	00.0
Auto. 60 min.	425	356	16.0
Auto. 60 min. + flexo wax	425	380	10.0
Auto. 60 min. + flexo wax + N_2	413	362	12.0
Auto. 30 min. + flexo wax + N_2	394	399	00.0
Auto. 15 min. + flexo wax + N_2	394	378	4.0
Auto. 5 min. + flexo wax + N_2	382	403	00.0
Dehydrated alfalfa			
Unheated	161	77	52.2
Unheated + flexo wax	161	141	12.4
Unheated + flexo wax + N_2	161	154	4.3
Autoclaved 10 min.			
No wax	147	99	32.6
Flexo waxed	147	131	10.9
Flexo waxed + N_2	147	160	00.0
Autoclaved 30 min.			
No wax	155	105	32.2
Flexo waxed	155	129	16.7
Flexo waxed + N_2	155	166	00.0

The results indicate a loss of carotene where the material was loose although washed with nitrogen and waxed. The result secured with sample no. 2 (table 3) was striking. No loss occurred when the material was unheated and only waxed but contained total moisture at a level of about 20 per cent. Further, the material had turned brown and had a very pleasant aroma. Perhaps the oxygen left in the material had combined with the chlorophyll and the chlorophyll had gone into the "brown" stage, giving an anaerobic

TABLE 3

Effect on carotene preservation of various treatments of dehydrated cereal grasses (oats) involving addition of 15 per cent of water

(Loose in cellophane tubes, kept at 22–25° C.)

Treatment	Color	Carotene content, µg./g. (air-dry basis)		% loss 3 mos.
		Initial	3 mos.	
Unheated—no wax	Green	380	189	49.7
Unheated—waxed	Brown	380	380	0.0
Unheated—N_2 washed—waxed	Brown	380	381	0.0
95° C.—40 min.	Green	418	285	32.0
95° C.—40 min.—waxed	Green	418	310	26.0
95° C.—40 min.—N_2 washed—waxed	Green	418	303	27.6

condition, or possibly the oxygen had been used up by tissue respiration or microorganisms with CO_2 production and O_2 consumption. At any rate, there was complete carotene preservation under a very simple procedure. Where the samples had been dried at 95° C. and the added water lost, the color remained green with considerable loss of carotene.

To secure more data on the behavior of sample 2 (table 3), an extended series of samples was prepared using both commercially dehydrated oats and dehydrated alfalfa.[3] Round cardboard boxes, 3.5 inches in diameter by 4 inches deep, were used as receptacles. The materials were mixed with varying percentages of water, firmly pressed by hand into the receptacles, and

TABLE 4

Effect of varying levels of added water on carotene preservation in dehydrated cereal grasses (oats) and alfalfa kept at 22–25° C.

Treatment	Color	Carotene content, µg./g. (corrected to original water basis)		% loss 3 mos.
		Initial	3 mos.	
Dehydrated oats—10.1 per cent initial water content				
No H_2O added—no wax	Green	379	278	26.6
No H_2O—waxed	Green	379	297	21.6
5% H_2O—waxed	Brown	379	386	00.0
10% H_2O—waxed	Brown	379	431	00.0
15% H_2O—waxed	Brown	379	387	00.0
15% H_2O—½″ head—waxed	Brown	379	410	00.0
Dehydrated alfalfa—7.2 per cent initial water content				
No H_2O added—no wax	Green	159	108	32.0
No H_2O added—waxed	Green	159	142	10.7
5% H_2O—waxed	Brown	159	153	3.7
10% H_2O—waxed	Brown	159	171	00.0
20% H_2O—waxed	Brown	159	171	00.0
20% H_2O—½″ head—waxed	Brown	159	174	00.0
30% H_2O—waxed	Brown	159	169	00.0

then covered tightly. In some cases a 0.5-inch head or air space above the material was left. Some of the alfalfa receptacles were covered completely with a thin layer of wax known as "Durex" and secured from the Dewey and Almy Chemical Company, Cambridge, Massachusetts. After 3 months of storage at room temperature (21–30° C.) the receptacles were opened and carotene determinations made. The results are shown in table 4.

All of the materials to which 5 per cent or more of water had been added turned brown in color. Those with the higher levels of water were deeper brown. The added water was in addition to that already present in the material, which was about 7 per cent in the alfalfa and 10 per cent in the oats. Where the color of the product was brown, a pleasant aroma not unlike

well-cured silage had developed. In all cases where 5 per cent or more of water had been added and the package covered with a wax to exclude free exchange of gases, the carotene was conserved completely for 3 months. There is no reason to believe that a longer time would have changed the results in this series.

There was no bulging of the boxes or evidence of internal gas pressure. However, there may have been a slow diffusion. The nature of the chemical changes and character of the gases produced (if any) were studied further. Where no water had been added, the material remained green in color but a considerable loss of carotene (20–30 per cent) had occurred. In many cases the amounts recorded as carotene were above the initial determinations. This phenomenon has been observed in studies on AIV silage (4) and on the effect of acids on carotenoids (5), and is attributed to the action of the acid on certain carotenoids with production of pigments of similar solubility to carotene. The amount of such pigments produced is relatively small. The fact that pigments of non-carotene nature may be produced by the action of acid on xanthophyll led to an examination of these samples for acidity. Surprisingly, the pH in all of the oat samples, including those where no change in color was observed, was 6.0. In the alfalfa samples it was 6.0–6.3. The higher figure was obtained with the alfalfa to which no water had been added. These figures represent an exceedingly low degree of free acidity.

The pH was determined by suspending 3 g. of the material in 25 cc. of water, stirring, and, after standing 20 minutes, reading on a pH meter. If there were acids produced the amounts must have been quite alike in all samples, mainly, CO_2. Even where a 0.5-inch head of air had been left in the receptacle, no loss of carotene occurred.

Further, to make certain that the observed increase in carotene did not represent an actual loss of carotene, with compensation by formation of other pigments, the carotene content was redetermined by the chromatogram. The method used was that outlined by Wilkes (6). The initial analysis of the alfalfa by the phasic method showed 159 μg./g. After storage for 4 months in the cold room ($-4°$ C.), it showed 143 μg. by the same method. By the chromatographic method 150 μg. of carotene were recovered where 5 per cent of water had been added and 138 μg. where 10 per cent of water was added and the materials kept at room temperature and sealed with flexo wax.

Similar results were secured with dehydrated oats where comparisons were made by the phasic and chromatographic methods. The original analysis of the dehydrated oats showed 379 μg./g. by the phasic method. After 4 months storage in the cold room ($-4°$ C.), it showed 385 μg. by the same method and 344 μg. by the chromatographic method. The experimental samples with 5 per cent of water, sealed with flexo wax and held at room temperature, showed 350 μg./g. by the chromatographic method and, where 15 per cent of water had been added and the samples likewise flexo-waxed

and held at room temperature, 360 μg./g. were obtained. If the thesis is accepted that by the chromatographic method more precise data are secured for the carotene content of a sample of dehydrated alfalfa or cereal grass than by the phasic method, then these data confirm the conclusion that practically no carotene is lost by the process of storage outlined. The increases observed under special storage with addition of water may be due, in part, to new pigments and also to limitations of the analytical methods.

To determine whether microorganisms were principally concerned in the reactions observed, samples of dehydrated oats and alfalfa were mixed with 10 per cent of water, placed in pasteboard containers 3.5 × 4 inches, lightly pressed by hand, and sealed with flexo wax. Controls with no additional water also were prepared. These samples were allowed to stand at room temperature for 5 days, then opened and assayed for bacterial count. The results follow:

	Color	Aroma	Bacterial count/g.
Dehydrated oats, control	No change	No change	2×10^4
Dehydrated oats, +10% water	"	Aroma more prominent	2×10^4
Dehydrated alfalfa, control	"	No change	6×10^4
Dehydrated alfalfa, +10% water	"	Aroma more prominent	4×10^4

The bacteria present were mostly aerobic spore-bearing bacilli, *Bacillus subtilis*. Very few staphylococci and very few mold spores were present.[4] The data indicate that the changes in the material containing added water were not primarily of bacterial origin, at least not in the first 5 days of the experiment.

It was important to determine whether carbon dioxide was being produced and oxygen used up where these dehydrated materials were mixed with added water, and further, whether the changes were occurring in the first 5 days after preparation. To this end 240 g. of dehydrated alfalfa as control (7.5 per cent water) and 240 g. with 10 per cent of water added were lightly packed in separate glass tubes, fitted with proper carbon dioxide guards, and set aside for 5 days at room temperature. At the end of this time the gases in the tubes were swept through weighed potash bulbs and the carbon dioxide determined. The control showed 8.4 mg. CO_2 while the alfalfa containing extra water showed 53.5 mg. CO_2.

In a somewhat similar experiment apparatus was designed to determine the amount of carbon dioxide and oxygen left in the air in contact with the alfalfa mass after standing at room temperature for 1, 3, 5, and 10 days. These experiments were conducted in glass tubes, where contact with outside

[4] We are indebted to Mrs. M. I. Robblee for this bacterial examination.

air was completely nullified. The air-dried material contained 7.5 per cent of water.

The results follow and are expressed in volume per cent of CO_2 and O_2 left in the atmosphere surrounding the alfalfa particles.

	CO_2	O_2
1 day	2.4	17.8
3 days	3.8	16.7
5 days	4.0	14.3
10 days	6.9	10.4

It is evident from the data that the process of respiration was comparatively slow, but was, nevertheless, a condition under which the carotene would be preserved. Just how complete a displacement of the O_2 with CO_2 is necessary for carotene preservation has not been determined.

Dehydrated plant tissue will vary in the rate of respiration, depending upon the temperature and time of drying, as illustrated in the following experiment. Alfalfa, cut on a University field, was spread on the laboratory floor and dried before a fan at a temperature of 22–25° C. This material was ground, lightly packed in glass tubes with and without added water (10 per cent), and the amount of CO_2 determined after standing 5 days at room temperature. The amount of material used in each experiment was 220 g. and contained an initial water content of 12.4 per cent. The air-dry sample (12.4 per cent water) produced 45 mg. of CO_2. The air-dry sample plus 10 per cent of water produced 144 mg. of CO_2. The amount of CO_2 produced where the water had been added represented, approximately, a concentration of 16 per cent of CO_2 in the gas mixture surrounding the alfalfa particles. It is apparent that this material had a respiratory rate appreciably greater than the commercially dehydrated product.

To determine what temperature changes would occur when the dehydrated products were stored with 10 per cent of added water, each of two large fiber cartons lined with paraffin paper was filled with approximately 28 lbs. of dehydrated alfalfa or dehydrated rye and sealed at the cover joint with paper. The material was lightly pressed into the cartons. Thermometers were inserted through the covers and sealed to prevent leakage of air. These cartons were held at room temperature of approximately 23.5° C. There was no observable temperature rise in either carton. Daily readings remained the same and at the end of 2 weeks both cartons recorded temperatures of 23.5° C., which was the room temperature. Theoretically, heat must have been a product of the respiratory changes in these masses but, apparently, the rate of production was so slow that through radiation the constant temperature prevailing in the room was maintained in the mass of material.

Smaller cartons filled with dehydrated alfalfa or rye (after addition of 10 per cent of water) and completely sealed with flexo wax, likewise showed no rise in temperature by the method used.

The data on gas production, bacterial activity, and temperature records

lend credence to the belief that tissue respiration is restored after addition of the water. In the dehydration process, not all, if any, of the respiratory enzymes were destroyed and, in the presence of added water and room temperature, their action was renewed. Their activity automatically creates an atmosphere of carbon dioxide with reduced oxygen tension, an ideal condition for carotene preservation.

DISCUSSION

The results secured in these studies emphasize the necessity of oxygen exclusion for carotene preservation in dehydrated cereal grasses or dehydrated alfalfa during storage. Destruction of the lipoxidase by autoclaving without further protection from access to air will not preserve the carotene.

The results secured by mere addition of water to the dehydrated material and then protecting against free access to air seem to offer practical procedures for carotene preservation in these materials. A total water content of 12 to 20 per cent, brought about by the addition of only 5 to 10 per cent of water, and the further protection against free access to air effectively stabilized the carotene for 3 months. In this process the material turned slightly brown and developed a very pleasant aroma. There may be objections to the use of a process where the green color partially is lost, but it should be emphasized that greenness is not always an assurance of a high nutritive value in these materials. At the present time there is no conclusive evidence that chlorophyll has any function in animal nutrition.

Whether the material could be prepared commercially with a final water content of 12–20 per cent has not been studied. Possible difficulties in grinding to a fine state would be encountered. If these materials could be so prepared and then stored under slight pressure in metal or fiber cartons or other containers so constructed as to prevent free exchange of air, it would seem probable that the carotene loss could be reduced considerably, if not prevented entirely. Such a process would save the adding of water after drying the material to a 6–10 per cent water content. The material to which only 5 per cent of water had been added was not so deep brown in color as when 10 per cent or more of water had been added. In fact, an olive green more nearly would describe the color. The nature of the chemical changes is not entirely clear. Oxygen absorption by the chlorophyll, with changes to the brown stage, appears possible but was unconfirmed experimentally. Acid products of a fixable character, such as lactic or acetic, must have been small in amounts and have served only as temporary intermediates.

Samples set up with added water and sealed from free access of air produced estimable quantities of carbon dioxide. Probably the reactions resulting from the addition of the water were those of tissue respiration, with utilization of the oxygen and production of carbon dioxide thus establishing partial, if not complete, anaerobic conditions. Under such conditions it would be expected that the carotene could be preserved.

Bacterial multiplication that could account for the chemical changes pro-

duced was not observed. The samples with added water showed no increase in bacterial numbers as compared with the controls in the first 5 days of the experiment; yet carbon dioxide was being produced.   It seems probable that the phenomenon observed was one of restored tissue respiration.

Practical application of this principle can be made by the use of containers allowing little or no diffusion of air or carbon dioxide.

It is possible, indeed probable, that the respiratory enzymes left in the dehydrated materials will vary with the temperature and the time the material has been exposed in the process of drying.   If this is true, the standardization of the drying method is necessary in order that a maximum of respiratory enzymes survive the process of drying and thus make possible the conservation of carotene by the method outlined.

SUMMARY

1. Destruction of the lipoxidase by autoclaving for 1 hour at 15 lbs. pressure did not preserve the carotene content of dehydrated alfalfa or cereal grasses exposed to air at room temperature.

2. The addition of 5 to 10 per cent of water to these dehydrated materials and then lightly packing them in receptacles sealed with flexo wax or Durex wax preserved the carotene completely for 3 months, when held at room temperature (22–25° C.).   The total water content for the oats was 15–20 per cent and for the alfalfa 12–17 per cent.

3. The process of preservation appears to be a restoration of more rapid respiratory enzyme action with utilization of the oxygen and formation of carbon dioxide.   There was no indication that bacterial action directly was responsible for the changes that occurred.   Temperature changes during the process of respiration were negligible in the masses of material used.

4. It is evident that receptacles with minimum or no air and CO_2 effusion rates are necessary for success in the preservation of the carotene by the method outlined.   Oxygen must be excluded or at least held at a low concentration.   This phase of the problem is under further study.

REFERENCES

(1) KON, S. K., AND THOMPSON, S. Y.   Factors Affecting the Stability and Estimation of Carotene in Artificially Dried Grass and Hays.   Jour. Agr. Sci., **30**: 622–638. 1940.

(2) MILLS, R. C., AND HART, E. B.   Studies on the Stabilization of Carotene in Dehydrated Feeds and Foods.   Jour. Dairy Sci., **28**: 1–13.   1945.

(3) MILLS, R. C., AND HART, E. B.   Further Studies on the Stabilization of Carotene in Dehydrated Alfalfa and Cereal Grasses.   Jour. Dairy Sci., **28**: 339.   1945.

(4) PETERSON, W. H., BOHSTEDT, G., BIRD, H. R., AND BEESON, W. M.   The Preparation and Nutritive Value of A.I.V. Silage for Dairy Cows.   Jour. Dairy Sci., **18**: 63–78.   1935.

(5) QUACKENBUSH, F. W., STEENBOCK, H., AND PETERSON, W. H.   The Effect of Acids on Carotenoids.   Amer. Chem. Soc. Jour., **60**: 2937.   1938.

(6) WILKES, J. B.   Chromatographic Determination of Carotene.   Indus. and Engin. Chem., Analyt. Ed., **18**: 702–707.   1946.

EFFECTS OF SHADE AND SPRINKLING WITH WATER ON SUMMER COMFORT OF JERSEY COWS

D. M. SEATH AND G. D. MILLER

Dairy Research Department, Louisiana Agricultural Experiment Station,
Baton Rouge

Results from a previous Louisiana test (7) have shown that dairy cows during warm weather spend a large portion of the daytime in the shade and that grazing time between morning and evening milkings may average less than 2 hours. After entering the shade of trees (average time 9 : 20 a.m.) respiration rates and body temperatures of cows showed a slow but gradual increase, with a maximum registered at 3 : 00 p.m., when cows entered the milking barn.

As explained by Rhoad (4), cows eliminate body heat by radiation and conduction of heat from their skin, and as latent heat of water vapor from skin and lungs. The portion of elimination by way of the lungs grows in importance as respiration increases, which in turn is most often caused by a rise in air temperature. At 71° F. Forbes, Braman, and Kriss (1) found that about 40 per cent of the heat left the cow's body as latent heat of water vapor. As reported by Kendall (3), the amount of water lost in this manner may vary as much as 12 lbs. per animal daily even when air temperature, feed, and other conditions are kept as uniform as possible. Each pound of moisture lost carries with it 1,086 B.T.U. of heat, he reports, but as air temperature drops there is a decrease in the amount of water eliminated by insensible perspiration.

Rhoad in his review (6) and report on experimental work (5) shows how time spent by cattle in the shade is associated with heat tolerance and that those with low tolerance, such as Angus, spend much more time in shade than do those of high tolerance, such as Brahman. He found Jersey cattle comparable to crossbreds carrying one fourth Brahman and three fourths Angus blood. Tests with these crossbreds on a hot day (air temperature 80–102° F.) showed a respiration rate of approximately 90 per minute while in the sun, with a drop to around 40 after 1 hour in the shade. Body temperatures were approximately 102.8° F. and 101.4° F., respectively, under these two conditions. Tests on a cooler day, with air temperature between 80 and 84° F., showed less change due to shade, *i.e.*, respiration rate was around 50 while cattle were in the sun, but dropped to 30 after 1 hour in the shade. Body temperature changed from approximately 101.7° F. in the sun to 101.0° F. in the shade.

Reports by Villegas (10) on use of an air-conditioned barn near Singapore with temperature kept at 70° F. showed Holstein cows averaging 24 lbs.

of milk daily as compared to 9 lbs. from cows in an open, well-ventilated barn exposed to tropical temperatures. Reproduction records showed that 58 per cent of cows in the air-conditioned barn conceived as compared to 25 per cent in the other barn.

Conversation with people who have visited the tropics has disclosed observations made by them where water in a pond or stream or from various water sprinkling devices was used to make milking animals more comfortable during hot days. This applies particularly to water buffalo and to a lesser extent to cattle. Observations in Louisiana (9) revealed that Holstein cows had body temperatures on warm days that averaged approximately 0.75° F. higher than did Jerseys within the same herd. A high percentage of these Holsteins sought relief by lying in water and mud whenever available, a practice not often followed by the Jersey cows.

EXPERIMENTAL

Four grade Jersey cows were observed over a period of 10 days in an attempt to determine how shade alone, or shade following sprinkling with water, affected milking cows removed from the effects of direct sun's rays during the summer. Bright sunshiny days were selected for making the tests; the first day of observation was on June 22, 1945, and the last one on August 1, 1945. Air temperatures in the shade varied from 83 to 90° F. and relative humidity from 61 to 80 per cent.

The reversal experimental design was used for the test. On each test day the cows were tied by halters to a fence located in the sunshine. They were left exposed to the direct rays of the sun between the hours of 12:00 noon and 2:00 p.m. Records then were made of rectal body temperatures, respirations as indicated by flank movements, and pulse rates as determined by placing tips of fingers on the underside of the tail and adjacent to the coccygeal artery. Following this, two of the cows were removed (dry) to the shade of a small barn (with numerous openings for ventilation) while the other two first were sprinkled thoroughly with water varying from 83 to 85° F. before being taken into the barn. The procedure was varied so that each pair of cows had 5 days when they entered shade without sprinkling (dry) and 5 days when they were sprinkled first before entering the shade of the barn. Sprinkling was performed by using a hand-type sprayer and thoroughly wetting all portions of the cow's body. Records were made of body temperature, pulse rate, and respiration rate 0.5 hour and 1 hour after cows entered the shade of the barn, using the same procedure as when cows were outside.

RESULTS

Body temperature. In each case shade alone, as provided by the barn, was effective in reducing materially the body temperatures of the dry cows. Mean reduction for 5-day records on individual cows after 0.5 hour in

shade (table 1) varied from 0.24, to 0.42° F. with an average of 0.34° F. After remaining in the shade 1 hour, the reductions varied from 0.58 to 0.88° F., with an average of 0.74° F. Thus, actual body temperature after 1 hour in the shade averaged 101.92° F. This was lower than after 0.5 hour by 0.4° F. Reductions after 1 hour were 118 per cent greater than at the half-hour period.

When cows were sprinkled prior to entering the shade of the barn, their mean body temperature reductions in 0.5 hour varied from 0.3 to 0.8° F. with an average of 0.54° F. After 1 hour in the shade the mean reduction for the four cows varied from 0.78 to 1.4° F. In this case the 1-hour-period

TABLE 1

Changes in body temperature caused by shade or shade plus water sprinkling
(5-day mean values for individual cows)

Cow no.	Shade alone trial			Sprinkling plus shade trial		
	Body temp. after 2 hr. in sun	Body temperature reduction		Body temp. after 2 hr. in sun	Body temperature reduction	
		After 0.5 hr. in shade	After 1 hr. in shade		Wet and in shade 0.5 hr.	Wet and in shade 1 hr.
	(°F.)	(°F.)	(°F.)	(°F.)	(°F.)	(°F.)
3	102.46	0.26	0.70	102.14	0.30	0.78
4	102.42	0.42	0.82	102.20	0.54	1.00
11	103.24	0.42	0.88	103.22	0.80	1.40
12	102.50	0.24	0.58	102.56	0.54	1.16
Av.	102.66	0.34	0.74	102.53	0.54	1.08

decreases averaged 1.08° F., or 100 per cent greater than at the end of 0.5 hour.

Cows sprinkled prior to entering shade averaged at the end of 0.5 hour 0.2° F. lower than when not sprinkled; and after 1 hour they were 0.34° F. lower. The advantage of sprinkling vs. not sprinkling at the end of 0.5 hour was 59 per cent, and at the end of 1 hour 46 per cent. Actual temperatures of sprinkled cows after 1 hour in the shade averaged 101.45° F., or well within the range of normal (2).

Respiration rate. After being in the shade (dry) for 0.5 hour, respiration rates reduced from an average of 83 to 55.8 per minute. Actual mean reductions due to shade alone (table 2) ranged from 22.6 to 32.6 among the four cows and averaged 27.2. With but one exception (cow no. 12), respiration rates were slightly faster at the end of one hour than at the half-hour period. The average reduction was 25.2 per minute, showing that cows were breathing two respirations per minute faster than at the half-hour period.

Cows when sprinkled prior to entering shade dropped much lower in respiration rates than when not sprinkled. Before sprinkling, respiration

TABLE 2

Changes in respiration rate caused by shade or shade plus water sprinkling
(5-day mean values for individual cows)

Cow no.	Shade alone trial			Sprinkling plus shade trial		
	Respira-tion after 2 hr. in sun	Respiration reduction		Respira-tion after 2 hr. in sun	Respiration reduction	
		After 0.5 hr. in shade	After 1 hr. in shade		Wet and in shade 0.5 hr.	Wet and in shade 1 hr.
	(*per min.*)	(*per min.*)	(*per min.*)	(*per min.*)	(*per min.*)	(*per min.*)
3	74.0	22.6	16.5	74.0	37.8	34.2
4	88.2	26.4	22.2	82.2	39.8	36.6
11	80.8	27.4	24.4	92.2	50.8	37.2
12	89.2	32.6	37.8	105.6	69.2	58.6
Av.	83.0	27.2	25.2	88.2	49.4	41.6

rates averaged 88.2 (table 2), while at end of 0.5 hour in shade they averaged 38.8, a reduction of 49.4. At the 1-hour period respirations had increased to an average of 46.6 or 7.8 per minute faster than at end of 0.5 hour. When compared to reductions in respiration rates of non-sprinkled cows, the reductions following sprinkling were 81 per cent greater at the half-hour period and 65 per cent greater at the hour period.

Pulse rates. Pulse rates appeared to change more slowly than did body temperatures or respiration rates. When dry cows entered the shade, they showed little change in pulse rate at the end of 0.5 hour (table 3). The average reduction from the original rate of 68.5 was only 0.8 per minute. Cow no. 11 actually averaged faster by 0.6 per minute. Shade alone at end of 1 hour effected a significant change in pulse rate, with mean reductions for cows varying from 1.0 to 5.8 and averaging 3.6 per minute.

Sprinkling of cows prior to entering shade resulted in a great reduction in pulse rate. After 0.5 hour in shade pulse rates had dropped an average

TABLE 3

Changes in pulse rate caused by shade or shade plus water sprinkling
(5-day mean values for individual cows)

Cow no.	Shade alone trial			Sprinkling plus shade trial		
	Pulse rate after 2 hr. in sun	Pulse rate reduction		Pulse rate after 2 hr. in sun	Pulse rate reduction	
		After 0.5 hr. in shade	After 1 hr. in shade		Wet and in shade 0.5 hr.	Wet and in shade 1 hr.
	(*per min.*)	(*per min.*)	(*per min.*)	(*per min.*)	(*per min.*)	(*per min.*)
3	71.8	1.8	5.8	67.6	7.8	7.6
4	69.0	0.6	1.0	64.8	5.8	3.8
11	69.6	− 0.6	5.8	71.0	2.8	7.0
12	63.6	1.2	2.0	68.6	8.0	7.9
Av.	68.5	0.8	3.6	68.0	6.1	6.6

of 6.1 per minute, and the reduction averaged 6.6 per minute 1 hour after entering shade, leaving actual pulse rate at 61.4 per minute, the lowest of any observation period.

DISCUSSION

In the present study cows entering shade (dry) derived more benefit from the shade furnished by the openly ventilated barn than did cows observed in a previous study (7) utilizing shade of trees in a pasture. It is probable that the barn furnished more complete shade than did the trees in the pasture. Also, air temperatures in the previous study continued to increase after cows entered shade (average time 9:20 a.m.); in this study there was little increase in air temperature after 2:00 p.m., when cows were taken from sunshine into shade. Another factor to consider is that cows used in the present study were all grade Jerseys producing only small amounts of milk. In the pasture study (7) heavier producing cows were observed and one-half of the cows were Holsteins, a breed shown (9) to have a lower rating on heat tolerance than Jerseys. It is possible that a higher humidity in the forenoon than in the afternoon (which is usual in Louisiana) may be a contributing factor to differences found in these two experiments. The effects due to this cause, however, would not be expected to be large, in view of results from a previous study (8), where it was found that high humidity played a minor rôle as a factor affecting body temperature, respiration rate, and pulse rate of dairy cows.

When cows in this study were sprinkled with water prior to entering shade, their body temperatures and respiration rates rapidly approached what has been reported as normal (2). It probably would require periodic sprinkling, perhaps once per hour, to hold such cows closer to normal than cows going into shade without sprinkling. However, as has been shown in this experiment, the unsprinkled group of cows would be slower in approaching normal. Whether or not practical sprinkling devices can be developed remains to be seen. Preliminary trials by the authors have shown that cows do not care to go into a coarse spray of water such as that produced by a conventional hose nozzle often used for lawns and gardens. Cows will go into a finer spray when located in the shade, according to a verbal report from a dairyman having had experience on a tropical island during the last war emergency period. Likewise, it has been observed by the authors that cattle when abnormally warm will usually relish wading into streams or ponds, particularly if they are located in the shade.

SUMMARY

1. Four grade Jersey cows were observed during 10 relatively warm days in an effort to determine how shade alone or sprinkling with water followed by shade affected their comfort. Air temperatures during periods of obser-

vation varied from 83 to 90° F. and relative humidity between 61 and 80 per cent.

2. Body temperatures of cows after exposure to sunshine for 2 hours averaged 102.66° F., and removal to shade (dry) resulted in reductions of 0.34° and 0.74° F. after 0.5 hour and 1 hour, respectively.

3. Sprinkling of cows (with original body temperatures after being in the sun of 102.53° F.) reduced body temperatures by 0.54° F. after 0.5 hour in shade, and by 1.08° F. after 1 hour in shade. In the latter case cows had temperatures which are considered normal.

4. Respiration rates reduced to levels which averaged lower after 0.5 hour than after 1 hour in shade. Cows not sprinkled showed a respiration rate of 83 per minute in the sun and an average decrease in rate of breathing of 27.2 at end of 0.5 hour and 25.2 after 1 hour in the shade.

5. Cows sprinkled prior to entering shade had much greater reductions in rate of breathing than did non-sprinkled cows, i.e., 49.4 less after 0.5 hour and 41.6 less after 1 hour.

6. Reductions in rate of breathing for non-sprinkled cows vs. those sprinkled favored the latter group by 81 per cent at end of 0.5 hour and 65 per cent after 1 hour in shade.

7. Average reduction in pulse rate for non-sprinkled cows after being in shade 0.5 hour was insignificant (0.8 per minute) but was significant (3.6) after 1 hour. Cows when sprinkled showed decreases in pulse rate that averaged 6.1 after 0.5 hour and 6.6 after 1 hour.

8. Either shade alone or sprinkling followed by shade was found effective in reducing body temperature, respiration rate, and pulse rate of dairy cows, with the second procedure being more rapid and also more effective in causing animals to approach readings which are considered normal.

REFERENCES

(1) FORBES, E. B., BRAMAN, W. W., AND KRISS, M. The Heat Production of Cattle in a Respiration Calorimeter as Related to the Rate of Ventilation and to the Moisture Content of the Air. Jour. Nutr., 5: 387–394. 1932.

(2) GAALAAS, R. F. Effect of Atmospheric Temperature on Body Temperature and Respiration Rate of Jersey Cattle. Jour. Dairy Sci., 28: 555–563. 1945.

(3) KENDALL, J. C. Perspiration a Factor in Animal Metabolism. N. H. Agr. Expt. Sta. Bul. 256, Ann. Rpt., 1930: 5–6. 1931.

(4) RHOAD, A. O. The Influence of Environmental Temperature on the Respiratory Rhythm of Dairy Cattle in the Tropics. Jour. Agr. Sci., 26: 36–44. 1936.

(5) RHOAD, A. O. Some Observations on the Response of Purebred Bos taurus and Bos indicus Cattle and Their Crossbred Types to Certain Conditions of the Environment. Amer. Soc. Anim. Prod. Proc., 1938: 284–295. 1938.

(6) RHOAD, A. O. Climate and Livestock Production. Yearbook of Agr., U. S. Dept. Agr., 1941: 508–516. 1941.

(7) SEATH, D. M., AND MILLER, G. D. Effect of Warm Weather on Grazing Performance of Milking Cows. Jour. Dairy Sci., 29: 199–206. 1946.

(8) SEATH, D. M., AND MILLER, G. D. The Relative Importance of High Temperature and High Humidity as Factors Influencing Respiration Rate, Body Temperature, and Pulse Rate of Dairy Cows. Jour. Dairy Sci., 29: 465–472. 1946.

(9) SEATH, D. M., AND MILLER, G. D. Heat Tolerance Comparisons between Jersey and Holstein Cows. Jour. Animal Sci., 6: 24–34. 1947.

(10) VILLEGAS, VALENTE. Livestock Industries of Cochin, China, Cambodia, Siam, and Malaya. Philippine Agr., 27: 693–725. 1939.

ETHYL ALCOHOL FROM WHEY

M. ROGOSA, H. H. BROWNE,[1] AND E. O. WHITTIER

*Division of Dairy Research Laboratories, Bureau of Dairy Industry, Agricultural
Research Administration, U. S. Department of Agriculture*

The preliminary report by Browne (1) on the production of ethyl alcohol from the fermentation of lactose in whey was published before the War. This early work with small batches of material gave evidence of promise. Whey as a raw material seemed a likely source of alcohol, since it probably is as cheap as any source of fermentable sugar, if a sufficient supply is readily and locally available. Furthermore, there is the constant problem, sometimes serious, of reducing the B.O.D. of effluents to streams. Therefore, work was conducted on the selection of desirable yeast strains, on the physiology of lactose-fermenting yeasts, of which practically nothing was known, and on the definition of the conditions for a more efficient and economically feasible fermentation of lactose to alcohol by yeast.

EXPERIMENTAL AND RESULTS

At the beginning of this work it was quickly apparent from the results being obtained with the very few strains of lactose-fermenting yeasts then available, that it was essential to examine many different types of lactose-fermenting yeasts for their suitability for alcohol production. Accordingly, a large and heterogeneous collection of lactose-fermenting yeasts was acquired.

The ability of the different types of lactose-fermenting yeasts to ferment the lactose in whey was measured by direct analyses of the residual lactose in the fermenting flasks after various periods of incubation at the optimal fermentation temperature for each organism. In each instance at least five analyses were made at different times during the course of a fermentation period. The relative rates of fermentation of whey containing 5 per cent of lactose at the beginning of the fermentation are shown in figure 1. After 55 hours *Torula cremoris* #2 had fermented all the lactose. In comparison, the following percentages of residual lactose were present in the case of other organisms tested: *Zygosaccharomyces lactis*, 3.8; *Torulopsis kefir*, 2.9; *Mycotorula lactis*, 3.1; *Candida pseudotropicalis*, 1.7; *Saccharomyces anamensis*, 1.6; *Saccharomyces lactis*, 1.7; *Saccharomyces fragilis*, 1.4; *Torula lactosa*, 1.3; *Torula sphaerica*, 1.2; and Type F, an unidentified lactose-fermenting yeast, 1.3. Thus, certain strains of *Torula cremoris* were the most efficient of all the tested lactose-fermenting yeasts in the fermentation of lactose in whey. Consequently, the later pilot plant experiments were conducted with a strain of this yeast, *Torula cremoris* #2.

[1] Deceased January 7, 1947.

It then seemed advisable to determine the optimal conditions for fermentation. Tests at temperatures of 30°, 32°, 34°, 37° and 42° C. were made in a number of experiments, the results of which are depicted in figure 2. Fermentation took place faster at 37° C. than at any of the other temperatures used. However, after slightly longer intervals of time than are shown in the figure, the initial and somewhat later superiority of 37° C. over 32° C.
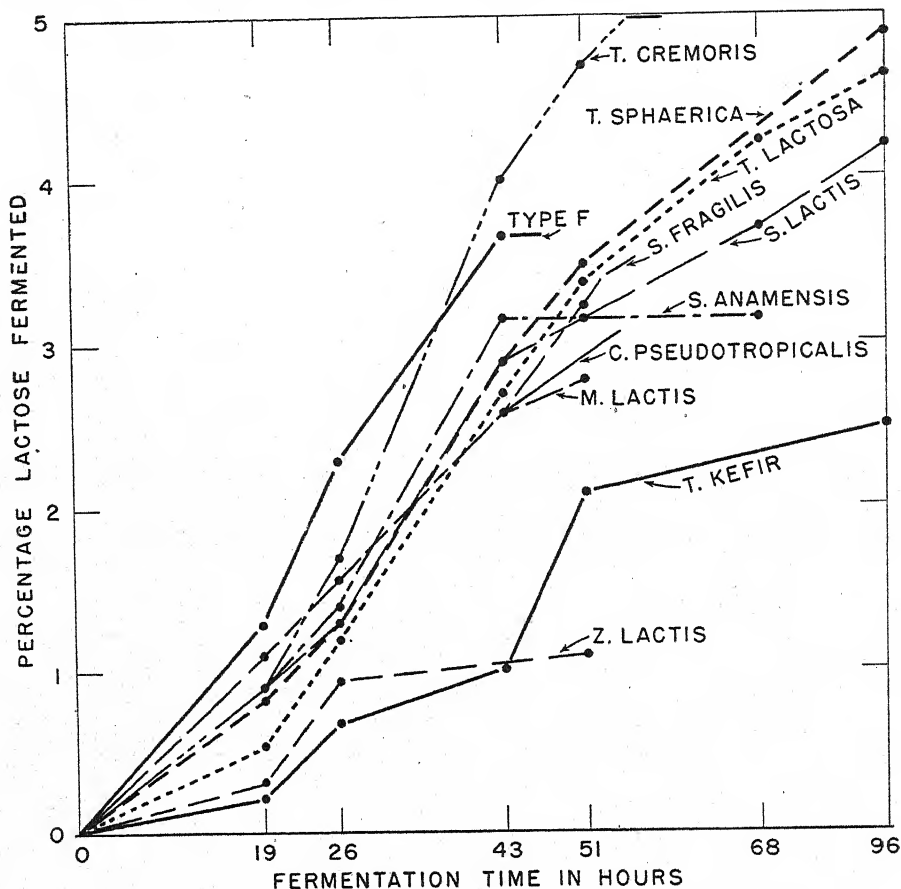
FIG. 1. Relative efficiency of lactose fermentation by lactose-fermenting yeasts.

is hardly apparent, and usually there is very little difference in the times necessary to effect complete fermentation (no residual lactose) at the two temperatures. Also, in fermenting larger batches of whey (150 gallons) which had an initial temperature of 30° C., the temperature rose to a maximum of 33–34° C. (heat of fermentation) and remained at this level throughout the most active period of the fermentation. Because of these considerations and also because higher temperatures may induce greater losses of

alcohol by evaporation, thus lowering the yield, it is recommended that a temperature range of 33–34° C. be used.

The question as to how much yeast should be used in the fermentation is important in terms of the time and economy of the fermentation. On the basis of the average number of grams of lactose fermented per gram of yeast per hour of elapsed fermentation time, it was established that a maximum amount of yeast corresponding to 2 per cent of the weight of the lactose initially present is sufficient to ensure a satisfactory rate of fermentation. In experiments with larger batches of whey, amounts of yeast as
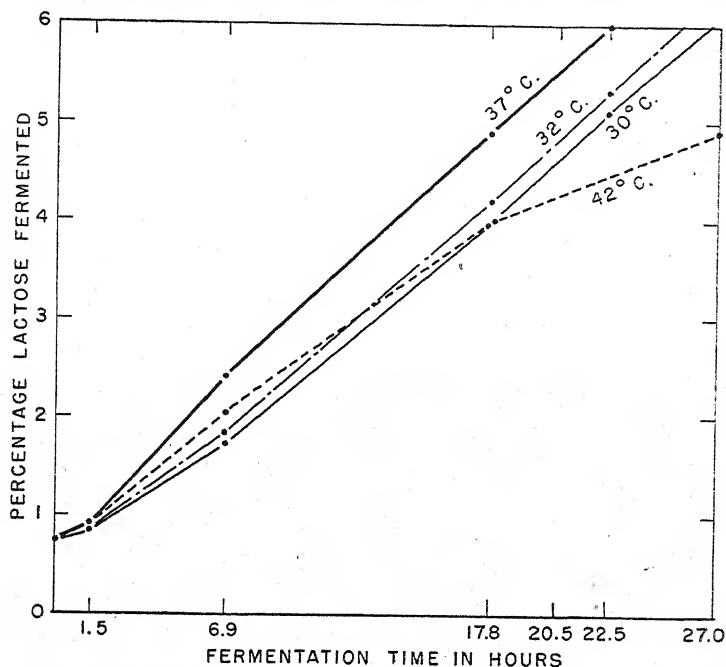
Fig. 2. Influence of temperature on the rate of lactose fermentation by *Torula cremoris.*

low as 1 per cent fermented the whey at a satisfactory rate if the yeast was in good condition.

The whey may be treated with heat, and either sour whey or acid may be added to precipitate the protein in the whey before the fermentation. If the whey is not treated, the initial pH of the whey mash should approximate a value of 6.0. If the whey is acidified, our experience with different batches and types of whey has been that the initial pH of the clarified whey should lie within a range of 4.8 to 5.2. Obviously, it is important to know what effects the initial pH, as well as the change in pH during the course of the fermentation, have on the rate and extent of lactose fermentation by the yeast. Also, it is desirable to conduct the fermentation at as low a

pH value as possible in order to minimize growth of contaminating micro-organisms. Clarification may be of great advantage regardless of the utility or economy of isolating the whey protein, since the pH is lowered.

Results of fermentation begun at different pH levels from 6.0 to 4.6 are depicted in figure 3. A pH range of 4.7 to 5.0 is satisfactory for a good fermentation. Peculiarly, an initial pH of 6.0 (unclarified whey) also was satisfactory, whereas at intermediate pH levels irregular and less satisfactory results were obtained.
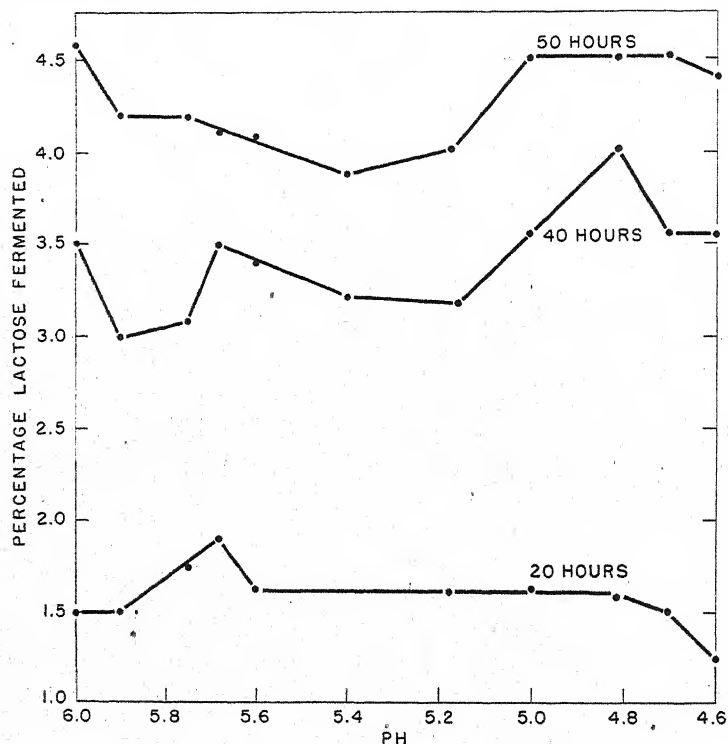
FIG. 3. Influence of pH on the fermentation of lactose by *Torula cremoris*.

The course of pH change from the initial value is shown in figure 4. A relatively small change in acidity occurs during the course of a fermentation when the fermentation is begun within the range of pH 4.7 to 5.0. In view of these results, it is recommended that the initial pH of a whey mash be adjusted to a range of 4.7 to 5.0.

Yields averaging 90.73 per cent of the lactose as alcohol have been obtained from the complete fermentation on a laboratory scale. Under semi-plant conditions yields were somewhat lower (as low as 84 per cent), probably due to the inefficient still employed. These yields compare favorably with other processes.

The quality of the alcohol was highly satisfactory. Customary "rub" tests for fusel oils and esterification tests for amyl alcohol were negative. Like other alcohols produced from grain and similar raw materials, this alcohol contains small quanties of aldehydes. However, they may be eliminated conveniently in the rectification of the crude distillate.

By-products from the fermentation, such as the whey protein and the slops, are of value as feed. The riboflavin and other vitamin content will
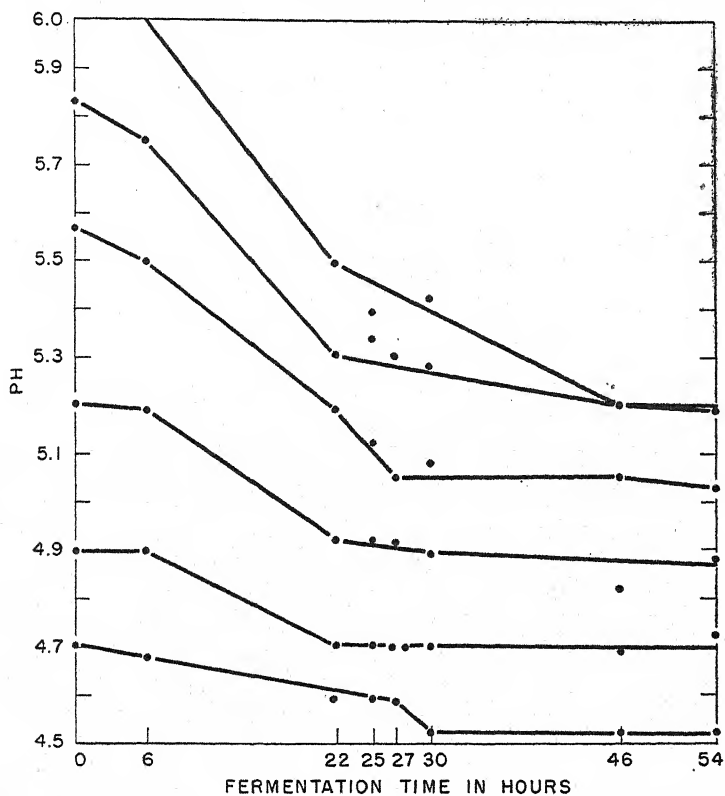
FIG. 4. Course of pH change during fermentation by *Torula cremoris*.

compare well, if not more favorably, with the vitamin content of the original whey on a dry basis. If the slops are to be dried for feed, the beer should be distilled at its naturally acid pH. Subsequent rectification of the crude alcohol can be conducted in the alkaline region to remove aldehydes.

EQUIPMENT AND OPERATION

On the basis of experimental work with 150-gallon batches of whey, it appears that the equipment for alcohol production should include at least the following items: a separator for removing the fat from the whey, a tank

fitted with a steam pipe for heating the whey, a filter press, a cooler for cooling the heated whey, one or more closed fermenting vats depending on the capacity of the plant, yeast tubs in a yeast room for propagating the yeast, an air line provided with a filter to furnish sterile air, a yeast recovery separator, an appropriately designed still, a storage tank for the distilled alcohol, condensing and drying facilities for the stillage slops if they are to be recovered, pumps, pipe lines, and a source of steam.

The accompanying flow-sheet (figure 5) gives a simplified and general
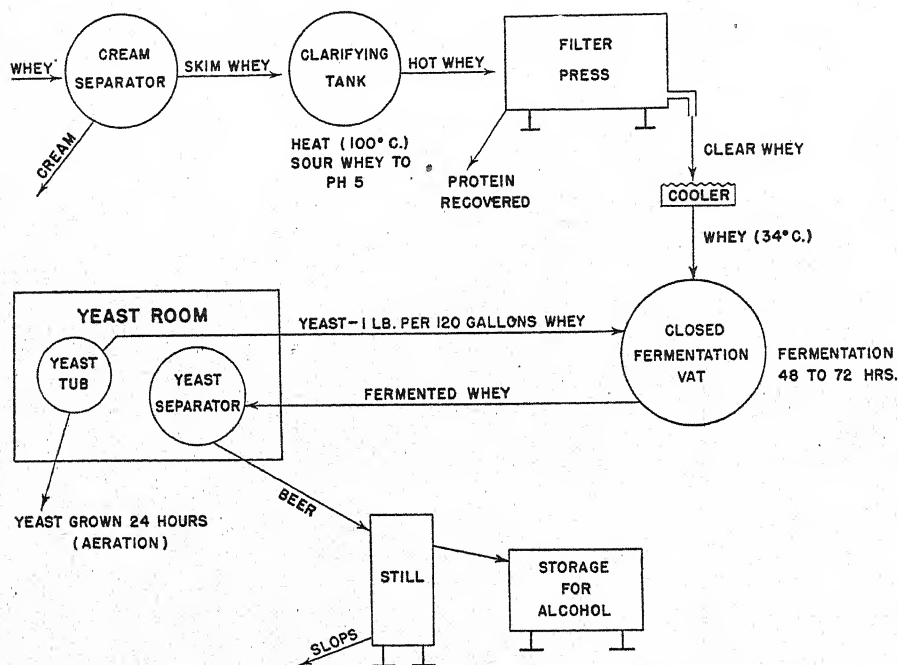
FIG. 5. Flow-sheet of alcohol production from whey.

picture of a total operation.   The steps in which a filter press and yeast recovery separator are used may be omitted. This is true particularly if the slops are to be recovered and sold for feed.   In this case all the protein in the whey and the yeast from the fermentation are wanted in the condensed or dried stillage slops for their nutritional value.

DISCUSSION

It is difficult to predict the costs involved compared to grain or molasses fermentation because much depends on local conditions, such as availability of raw materials; nevertheless, it is possible to make some tentative statements. Capital costs may compare favorably with other processes, since the alcohol plant could be a by-product plant operating as an adjunct to a

cheese or casein factory from which some space, equipment, and low-pressure steam would be available. In this case, the major capital outlay would be limited to the cost of clarifying, fermenting, condensing, and distilling equipment. The steam cost for a finished 95 per cent alcohol from whey would be higher than that from other processes, since a grain or molasses mash can be fermented to a higher alcohol content. But the relative cost of capital outlay, raw materials, and similar items probably would compensate for the higher steam costs of the whey process. However, higher steam costs might be reduced considerably and even to a favorable competitive basis because sugar (glucose sirups or molasses) might be added to the whey and fermented to a higher alcohol content than the lactose naturally present in whey would permit. The addition of other sugars is feasible since whey contains all the necessary growth factors for the fermentation of these sugars by lactose-fermenting yeasts. Since the supply of whey may not be steady, and since the plant should be constructed for a maximum supply of whey, the addition of sugar sirups seems to be an economically desirable feature of the process.

SUMMARY

1. Various types of lactose-fermenting yeast were tested for their efficiency in fermenting lactose in whey, and *Torula cremoris* was selected as the most efficient organism.

2. The optimal operating temperature for the fermentation in the semiplant was found to be 33–34° C.

3. The pH of a whey mash should be within the range of 4.7 to 5.0.

4. Yeast equivalent to two per cent of the weight of the lactose is sufficient for satisfactory fermentation.

5. Yields of alcohol were obtained averaging 90.73 per cent in the laboratory and as low as 84 per cent in the plant.

6. The equipment and the operation of a plant producing alcohol from whey are described.

7. The relative economy of the process is discussed.

REFERENCE

(1) BROWNE, H. H. Ethyl Alcohol from Fermentation of Lactose in Whey. Indus. and Engin. Chem., News Ed., 19: 1271. 1941.

ASSOCIATION ANNOUNCEMENTS

ANNUAL MEETING: ONTARIO AGRICULTURAL COLLEGE, GUELPH, ONTARIO, CANADA, JUNE 24–26, 1947

ABSTRACTS OF PAPERS

All abstracts of papers to be given at the annual meeting must be received by the section Chairman by May 30. They should be mailed to the committee chairman to whom the title was sent.

REGISTRATION AND HOUSING

Registration headquarters will be in the Administration Building, Ontario Agricultural College, Guelph, Ontario.

Housing facilities will be available in College dormitories. Rooms in local hotels are very scarce. Meals will be served cafeteria style in Creelman Hall. An attempt will be made to house family groups in the same dormitories. Rooms and meals will cost $2.50 a day. Individual meals for delegates not in the dormitories will cost 50¢ each. A return card relative to advance registration and housing will be sent to members by the Association Secretary in May.

PROJECTION EQUIPMENT

Lanterns will be available in all lecture rooms for projection of standard and $2'' \times 2''$ slides. Projectors for 16-mm. movies will be available by arrangement. Request for projection equipment should be made at the time abstracts of papers are submitted to the respective section Chairman. For the benefit of any bringing special electrical equipment, the available current is all 25 cycle.

COMMITTEE MEETINGS

Those wishing rooms for Extension and Production Section Committee meetings should write or contact G. E. Raithby and those in the Manufacturing Section wishing the use of rooms for Committee meetings should write or contact W. H. Sproule.

SPECIAL MEETINGS

Groups wishing rooms and equipment for special meetings before, during, or after the regular session will please contact G. E. Raithby. Provision can also be made for a limited number of breakfasts, luncheons, or dinners for special groups.

TRAVEL SUGGESTIONS

Guelph is serviced by the two main railway lines in Canada, Canadian National and Canadian Pacific. Bus lines from the South, East and West

271

lead to the city.   Highways nos. 6, 7, and 24 pass through the city, and motorists should make inquiry after crossing the border for highway suggestions.   Representatives of the Ontario Tourists Association, located at border crossing points in Ontario, will be notified of the Conference and will be glad to give assistance.   The nearest airport is at Malton, Ontario, about fifty miles from the College.   Malton is on the main line of the Canadian National Railway between Toronto and Guelph.

RECREATION

On the campus are tennis courts, baseball diamonds, and an indoor swimming pool.   Adjoining the College is a pay-as-you-play golf course.

G. E. Raithby of the Animal Husbandry Department, Ontario Agricultural College, Guelph, has been designated as the representative of the host institution.

W. D. Tolton of the Ontario Agricultural College, Guelph, has been appointed to membership on the Extension Section Committee on Teaching Methods and Exhibits.

WITHIN–COW REGRESSION OF MILK–ENERGY YIELD ON AGE AND LIVEWEIGHT[1]

W. L. GAINES

Illinois Agricultural Experiment Station, Urbana

H. P. DAVIS AND R. F. MORGAN

Nebraska Agricultural Experiment Station, Lincoln

INTRODUCTION

Certain DHIA records of cows in Illinois farm herds (3, 4) showed that milk-energy yield is practically unaffected by age of cow, independent of liveweight. On the other hand, milk-energy yield is greatly affected by live-weight, independent of age. These results relate to Holstein and Jersey cows, separately by breed. In each breed some of the cows were registered animals and some were not.

A similar result was found (1) from certain more accurate records (*e.g.*, milk weighed at each milking instead of one day per month) in the Nebraska Station herd at Lincoln. The Nebraska data are for registered cows of the Ayrshire, Guernsey, Holstein, and Jersey breeds, all treated as one group. The lumping of the data for the four breeds quite possibly could disturb the general validity of the result, because the Holsteins were markedly larger than the other breeds and at·the same time had a decidedly higher milk-energy yield per unit liveweight than the other breeds. The present paper presents a refinement of the analysis for the Nebraska data by finding the age-weight-yield relation within cow for the Holstein breed.

PROCEDURE AND RESULTS

The general principle of statistical procedure used is the same as that used by Dickerson (2) in finding the within-cow regression of milk-fat yield on age (liveweight ignored). The present procedure involves fitting a three-constant equation to the observations; accordingly, only cows with three or

273

more lactations are used. The data provide 57 cows, each with 3 or more lactations, a total of 231 lactations distributed as follows:

Lactations	3	4	5	6	7	8	9
Cows	25	15	11	3	2	0	1

The following within-cow, within-breed, within-herd equations emerge, by least squares:

$$W = 917.4 + 9.822\,A - 0.04048\,A^2 \tag{a}$$
$$FCM = -1.69 + (0.0413 \pm 0.0045)\,W + (0.001 \pm 0.022)\,A \tag{b}$$
$$1,000\,FCM/W = 38.30 + (0.0012 \pm 0.0032)\,W + (0.004 \pm 0.016)\,A \tag{c}$$
$$1,000\,FCM/W = 31.00 + (0.245 \pm 0.044)\,A - (0.00142 \pm 0.00026)\,A^2 \tag{d}$$

W is liveweight in pounds, the average of three scale weighings on successive days within 31 days after calving. This particular stage of lactation for the measurement of liveweight is an essential feature of the present philosophy. Class units of one pound were used in the computations.

A is conceptual age of cow at calving in months, reckoned as $10 +$ birth age at calving in months, with all fractions dropped. The record of birth age at calving is accurate to a day but class units of one month were used in the computations.

FCM is milk-energy yield for the 8-month partial lactation in pounds of 4 per cent milk per day. The 8-month (243-day) partial lactation is used to avoid complications of advanced pregnancy. FCM is based on milk weights at each milking and monthly determinations of fat percentage. Class units of 0.1 lb. were used for FCM and $1,000\,FCM/W$ in the computations.[2]

W, A, FCM, and $1,000\,FCM/W$ are known for each lactation of each cow, and the equations deal with these items in the same lactation. For example, FCM is related to W of the same lactation, not W of some other lactation. The mean and range for the 231 lactations are as follows:

	A	A^2	W	FCM	$1,000\,FCM/W$
Mean	72	5,920	1,385	55.6	40.2
Range	35–174	1,225–30,276	979–1,854	31.9–80.7	25.4–56.4

DISCUSSION

Accepting the observations and calculations as of a satisfactory order of accuracy, what biological interpretations are warranted by the equations?

[2] $FCM = 0.4 \times$ milk $+ 15 \times$ milk fat, all in the same unit of weight. One pound $FCM =$ 340 kilocalories of milk energy. The correlation between milk-energy yield in calories determined by use of direct calorimetry and milk-energy yield in calories estimated by use of the FCM formulas is of the order 0.997. If the milk-yield and fat-yield data are valid, the estimate of milk-energy yield in terms of 4 per cent milk by the FCM formula also is valid. FCM is a technic for estimating milk energy. It is not a correction for fat percentage in the same sense that mature equivalent is a correction for age.

In the first place the equations are within cow (and, by physical restriction, within breed and within herd), which should enhance their biological meaning.

By equation (a) $W = 1,212$ at $A = 35$, reaches a maximum of 1,513 at $A = 121$, and then declines to 1,401 at $A = 174$. (Birth age is approximately 9.5 months less than A.) Holsteins are a large breed and the present 57 Holsteins are large animals of the breed. The declining phase of equation (a) is not very reliable because of a sparse population at values of A beyond 121. Taken at face value, it may indicate a lack of condition (fatness) at calving at advanced ages. Also, the equation is bound to be affected by the individuality of the few cows having lactations at advanced ages.

Equation (b) expresses FCM as a function of W and A. The coefficient of W is nine times as large as its standard error and highly significant in a probability sense. On the other hand, the coefficient of A is smaller than its standard error, *i.e.*, it does not differ significantly from zero. According to equation (b), an increase of 100 lbs. in liveweight is accompanied by an increase of 4.13 in FCM, independent of age, which is 344 times as great as the increase in FCM which accompanies an increase of one year in age, independent of liveweight. One pound of liveweight is more potent than 3 years in age, by the equation.

This within-cow result is in general agreement with the previous findings (1, 3, 4) that milk-energy yield is influenced profoundly by liveweight, independent of age, and is substantially unaffected by age, independent of liveweight. If these are the true relations, then the system of correcting milk yield for age of cow at calving is biologically unsound and should be superseded by a system based on liveweight within 31 days after calving.

The foregoing statement is not intended to deny the statistical validity of age correction where W is unknown, because age and liveweight are to some extent correlated. In the present study of 231 lactations, the correlation between A and W is 0.48 in total, 0.31 between cows, and 0.62 within cow. But this does not alter the biological situation. Age represents time, which is required for the organism to attain size (liveweight). The procedure of equation (b) is to allow age and liveweight to take their natural values and depend on the liveweight and age terms of the equation, adjusted by least squares to the observations within cow, to segregate the independent influence of age and liveweight. This procedure allows both age and liveweight to express themselves in a natural way. If age is restricted to first lactations, the range of liveweight is restricted because the animals have not had time to grow fully and, of course, the within-cow relationships cannot be ascertained at all.

Equation (c) proceeds to the next logical step, namely, since equation (b) indicates that FCM is largely a multiple of W, with the other two terms of little consequence, FCM/W should be largely independent of both age

and liveweight. Equation (c) shows that such is the case. The coefficients of W and A are smaller than their respective standard errors. Neither one is significantly different from zero.

Milk-energy yield per 1,000 lbs. liveweight, 1,000 FCM/W, is here regarded as a quantitative measure of lactational drive, or the intensity of lactation metabolism, on the assumption that lactation metabolism is proportional to milk-energy yield and the amount of protoplasm (work stuff) involved in lactation is proportional to liveweight.[3]

FCM/W stands in its own right as a factual measure of lactational drive. It is not to be regarded merely, or primarily, as a substitute for age correction. It is, rather, a direct biological measurement which has no need of age correction. Clearly, the lactational-drive philosophy is very different from the mature-equivalent philosophy.

In a similar way FCM/W is not to be regarded as a correction for weight. It is, again, a factual biological measurement which has no need of weight correction. The evidence substantiating this statement is satisfactory as within breed (Holstein or Jersey) but as yet is not conclusive as between breeds (Holstein and Jersey). When all the evidence is collected, we may find FCM/W is greater for Jerseys than for Holsteins (as FCM/W is unmistakably greater for dairy goats than it is for dairy cows). On the other hand, FCM/W, as used here, may prove to be a biologically equitable measure of lactational drive or dairy development between dairy breeds as well as within breed. From this standpoint FCM/W should appeal to those agencies working with the dairy breeds collectively, as producers of milk.

Equation (d) requires a slight modification of the discussion under equation (c). In equation (c), FCM/W is expressed as a linear function of W and A, while in equation (d), FCM/W is expressed as a curvilinear function of A alone by introducing a term in A^2. The coefficients of A and A^2 both are significant as judged by their standard errors. By equation (d) 1,000 $FCM/W = 37.8$ at $A = 35$, reaches a maximum of 41.6 at $A = 86$, and then declines to 30.6 at $A = 174$. As in the case of W in equation (a), the declining phase is not very reliable and is of little practical importance because of the infrequent occurrence of lactations very far advanced on the descending limb of the curve. The descending limb is of some theoretical interest as indicating senescence in the intensity of lactation metabolism in old age.

[3] Generally speaking, it is permissible to say that the amount of body protein in cows of different liveweights is proportional to liveweight. But there is a good deal of assumption in saying that the amount of protoplasm involved in lactation is proportional to W as here defined. The assumption is encouraged by the fact that milk-energy yield is proportional to W in the present data, according to the fitted equations (b) and (c). It must be recognized, however, that W is affected by fatness (a body food reserve available for lactation needs) as well as by size in the sense of body dimensions. The problem is complicated. However, the weight of visceral organs need not be a factor of great importance because the visceral organs have a wide margin of safety above the point of being a limiting factor in the amount of lactation.

Equation (d) indicates that the regression of FCM/W on age is curvilinear and significant in the probability sense. Can the increase of 3.8 in 1,000 FCM/W from the youngest age to age of maximum be ignored? In consideration of this question it is of interest to see how age-corrected FCM behaves in relation to age in the same 231 lactations. Applying the official age-correction factors of the Holstein-Friesian Association to each of the 231 lactations, the following within-cow equation emerges:

$$A-CFCM = 49.76 + (0.260 \pm 0.069)\ A - (0.00154 \pm 0.00040)\ A^2 \quad (e)$$

Equation (e) shows that the official age-correction factors do not completely remove age changes in FCM yield for these 231 lactations. According to the equation, age-corrected $FCM = 57.0$ at $A = 35$, increases to 60.8 at $A = 85$, and then decreases to 48.5 at $A = 174$. Comparison of equations (d) and (e) shows that FCM/W is practically as close to being independent of age as is age-corrected FCM for these 231 lactations, using the official age-correction factors.

The derivation of age-correction factors has long been a favorite occupation of investigators engaged with the biometric analysis of milk records. Following are two equations derived from the present 231 lactations:

$$\text{In total,} \qquad FCM = 23.26 + 0.7252\ A - 0.0033548\ A^2 \qquad (f)$$
$$\text{Within cow,} \qquad FCM = 24.42 + 0.7296\ A - 0.0036064\ A^2 \qquad (g)$$

Equation (f), dealing with the 231 lactations in total (the usual method of approach), indicates an age-correction factor of 1.35 for $A = 35$ (birth age 25.5 months). Equation (g), dealing with lactations within cow (theoretically a more refined method of approach), indicates an age-correction factor of 1.40 for $A = 35$. The official factor is 1.25.

Equation (g) appears to be a better way of deriving the age-yield relation generalized for the same cow than is the ratio method of Sanders (5), but the authors have no desire to add to the multiplicity of age-correction factors now in the literature. Consequently, use of lactational drive, measured as 1,000 FCM/W which has no need of age correction (and no need of weight correction within breed or probably within species), is advocated.

SUMMARY AND CONCLUSIONS

This paper deals with 57 registered Holstein cows in the Nebraska Station herd at Lincoln, Nebraska, each cow having three or more lactations, a total of 231 lactations. Age at calving (A), liveweight within 31 days after calving (W), milk-energy yield per day for the 243-day partial lactation (FCM), and lactational drive (1,000 FCM/W) are accurately known for each lactation of each cow. Various equations have been fitted by least squares to the observations, both in total and within cow. The within-cow

equations are presumed to give the best insight of the relationships between the variables from a biological point of view.

When FCM is expressed as a linear function of W and A, within cow, the coefficient of W is relatively large and highly significant while that of A is very small and not significantly different from zero. The constant term is very small. The independent effect on FCM of one pound in W is equal to the independent effect of 3.44 years in A. From this within-cow result it is concluded that for these 231 lactations, dealing with A, W and FCM as they actually exist under natural conditions, it is biologically unsound to correct yield for age because age has no effect on yield independent of live-weight.

When lactational drive (1,000 FCM/W) is expressed as a linear function of W and A, the constant term is large and the coefficients of W and A both are small, each being smaller than its own standard error, or neither one is significantly different from zero. From this within-cow result it is concluded that lactational drive (1,000 FCM/W, as defined) is a directly comparable measure of dairy development or intensity of lactation metabolism within these 231 lactations, being completely independent of both age and live-weight.

From the present within-cow equations, from previous equations, and from a metabolic or dynamic point of view, the conclusion is reached that lactational drive (as defined) affords a biological common denominator for dairy cattle as a whole, with respect to yield of milk.

REFERENCES

(1) DAVIS, H. P., MORGAN, R. F., AND GAINES, W. L. Live Weight and Milk-Energy Yield in the Nebraska Station Dairy Herd. Jour. Dairy Sci., 26: 625–641. 1943.

(2) DICKERSON, G. E. Estimates of Producing Ability in Dairy Cattle. Jour. Agr. Res., 61: 561–586. 1940.

(3) GAINES, W. L., RHODE, C. S., AND CASH, J. G. Age, Live Weight and Milk-Energy Yield in Illinois Cows. Jour. Dairy Sci., 23: 1031–1043. 1940.

(4) GAINES, W. L., RHODE, C. S., AND CASH, J. G. Age, Live Weight and Milk-Energy Yield—a Correction. Jour. Dairy Sci., 25: 15–18. 1942.

(5) SANDERS, H. G. The Variations in Milk Yields Caused by Season of the Year, Service, Age, and Dry Period. Part III. Age. Jour. Agr. Sci., 18: 46–67. 1928.

EFFECTS OF HIGH VITAMIN A INTAKE ON MILK AND FAT YIELDS AND ON VITAMIN A CONSTITUENTS IN MILK, BLOOD, AND LIVERS OF DAIRY COWS[1]

G. H. WISE AND F. W. ATKESON

Department of Dairy Husbandry

M. J. CALDWELL, D. B. PARRISH, AND J. S. HUGHES

Department of Chemistry

Kansas Agricultural Experiment Station, Manhattan

Recognition of the importance of vitamin A constituents in the diets of dairy cattle has resulted in extensive investigation of the effects of various vitamin A supplements on the health of the cow and on the properties of milk. A summary (2) of reports on the effects of feeding crude cod-liver oil and menhaden fish oil to lactating cows indicates that when these oils are given in sufficient quantities to augment the vitamin A potency of the milk, the percentage of fat is reduced. In recent years the feeding of vitamin A supplements such as shark liver oil and vitamin A concentrates has come into common use for certain classes of livestock. The addition of these high potency vitamin A materials to the diets of cows has increased, in varying degrees, the concentrations of this vitamin in the blood (5, 8, 9, 15), in the milk (1, 2, 7, 8, 10, 15, 18, 21), and in the liver (6), but has produced discrepant effects on yields of milk and of fat (1, 2, 7, 8, 10, 15, 18, 21, 23, 24).

The variability and the diversity of the results reported warranted further study of the effects of feeding vitamin A supplements to dairy cows maintained in a good state of nutrition. Hence, in an investigation designed to ascertain the effects of prolonged supplementation of massive amounts of vitamin A on the course of mastitis, additional observations were made on the yields of milk, the percentage of fat, and the concentrations of vitamin A in the milk, the blood, and the liver. The results from this phase of the investigation are reported herein.

EXPERIMENTAL PROCEDURES AND RESULTS

Grouping and Care of Experimental Animals

Experimental cows. Two comparable groups of dairy cows, the control and the supplemented, were used in this trial. The following factors, in the order listed, were considered in grouping the cows: breed, mastitic history, daily milk yields, stages of gestation and lactation, and body weights. Each group at the beginning of the trial consisted of nine mature cows, two Ayrshires, two Guernseys, and five Holsteins. Six of the cows in each group were lactating, being past the stage of peak production but not sufficiently

advanced in gestation to accentuate the rate of decline in yield; the remaining three cows, two Ayrshires and a Holstein, were in the early stages of the dry rest period, from 44 to 52 days prepartal. These dry cows were included to determine the effects of prepartal supplementation on postpartum changes.

In addition, two non-lactating Ayrshire cows in the last month of gestation were used to study in detail the effects of.level of vitamin A intake on the changes in carotenoids and vitamin A of the serum during the terminal stages of gestation and early period of lactation. The two cows had practically the same carotenoid and vitamin A content of the serum before they were subjected to experimental conditions.

Feeding and management. Prior to the initiation of the trial and throughout the experimental period of 12 weeks, during the months of November, December, and January, all cows of the two groups received a standard milking herd ration consisting of a 16 per cent protein concentrate mixture, Atlas sorgo silage, and alfalfa hay. The carotenoid content of the hay, on the moisture-free basis, ranged from 0.06 mg./g. in the early part of the trial to 0.03 mg./g. in the latter. The lactating cows were fed daily 1 lb. of concentrate mixture for each 4 lbs. of 4 per cent fat-corrected milk, 20 to 25 lbs. of silage per 1,000 lbs. body weight, and hay *ad lib.* The dry cows were fed daily 8 lbs. of the concentrate mixture per 1,000 lbs. of body weight and the roughages, the same as for the lactating cows. In addition to the barn feeds, the cows of the two major groups grazed on rye pasture 30 to 40 days before initiation of the experiment and 16 days following.

Throughout the experimental period, all cows (both the dry and the lactating) in the supplemented group received daily 1,250,000 USP units of vitamin A in a powdered medium.[2] Since this vehicle, described as a "soybean oil meal like" product, supplied nutrients in addition to vitamin A, the cows of the control group received soybean oil meal in quantities equal to the amount of vitamin A supplement fed to the other group. These additional feeds and supplements were given once daily in combination with the concentrate mixture. Considerable quantities of the vitamin A supplemented mixture were refused during the first several days of feeding, but after the cows became accustomed to the foreign flavor, no consumption difficulties were encountered.

All cows were subjected to standard herd management, which included feeding and milking twice daily, exercise whenever weather conditions permitted, and free access to water, common salt and hay in the same paddock.

Yields of Milk and Concentrations of Fat and of Vitamin A Constituents

Milk yields and fat percentages. Detailed records of the milk yields of individual cows were made throughout the experimental period. Samples

2 "Dry vitamin A", having 2,500–2,700 USP units per gram.

of milk were collected during two consecutive milkings each week for the determination of fat concentration by means of the standard Babcock procedure. The weekly milk and fat yields of the respective groups were sum-
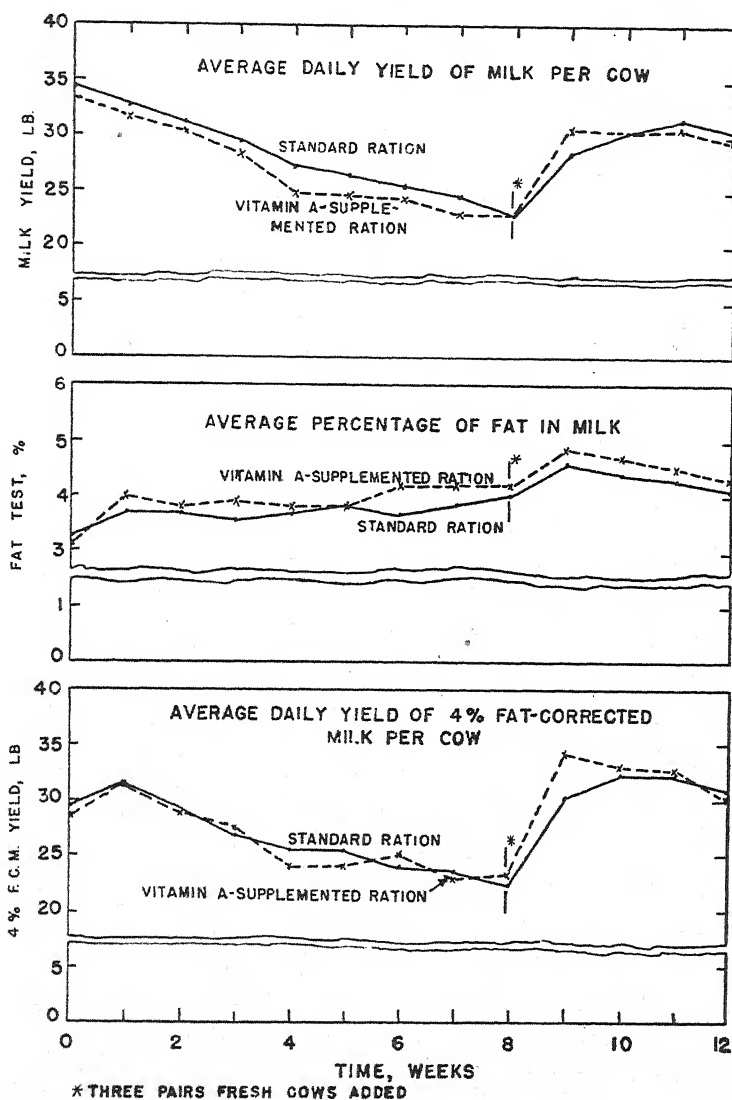
FIG. 1. The effects of dietary supplementation of vitamin A on milk yields and on fat percentages.

marized and subsequently converted to a 4 per cent fat-corrected basis according to the Gaines (11) formula. The yields of milk and fat (fig. 1) revealed no marked differences that could be ascribed to the rations fed.

Initiation of vitamin A supplementation during the early stages of the dry rest period revealed no advantage in production during the first month of lactation. Thus it appears that the daily addition of massive amounts of "dry vitamin A" to the dairy ration used in this experiment neither augmented total milk yield nor suppressed the fat percentage of the milk.

Concentration of carotenoids and vitamin A in the milk fat. During the terminal week of the experiment, a 1-day composite sample of milk was collected from each of seven individual cows in the respective groups for assays of carotenoids and vitamin A. (Milk from two pairs of cows was excluded because of mastitis complications.)

The analytical method employed was a modification of the double extraction procedure of Boyer *et al.* (4). A total of 70 ml. of ether was used in extracting vitamin A and carotenoids, and the volumes of wash solutions were adjusted accordingly. The washed solution of extracted vitamin A

TABLE 1

Average concentrations of vitamin A and carotenoids in the milk fat of cows on different levels of vitamin A intake

Daily supplement of vitamin A	Breed of cows	No. of cows	Vit. A and carotenoids of milk fat	
			Vitamin A	Carotenoids
None	Guernsey	2	$\gamma/g.$ 6.4	$\gamma/g.$ 5.8
	Holstein	3	7.3	3.4
	Ayrshire	2	7.8	4.1
1,250,000 USP units	Guernsey	2	24.1	2.5
	Holstein	3	30.5	4.1
	Ayrshire	2	36.1	1.9

and carotenoids was dried by means of anhydrous sodium sulphate, and the ether was removed from the extract by suction while heating in a water bath at 50–60° C. The residue was dissolved in 15 ml. of Skellysolve B. A 10-ml. aliquot was used for the final determination of carotenoids and of vitamin A. Shaking the ether solution with 5 ml. of a saturated solution of sodium chloride, as outlined in the original method, was omitted. Photometric measurements were made on a Coleman spectrophotometer, model 11, modified to reduce light intensity. Since the fat percentage in the samples was variable, the carotenoid and the vitamin A values were expressed as concentration per unit of milk fat.

The average, by breeds, of vitamin A in the milk fat from supplemented cows was approximately four times higher than that from the control cows. The carotenoids throughout were low, but, in accord with other reports (2, 8, 9, 10, 15), tended to be lower in the milk fat from vitamin A supplemented cows. There was, however, an exception in the case of two of the three pairs of cows of the Holstein breed; hence the average for this breed did not reveal

the reduction (table 1).   Since preliminary assays were not made, individual differences could account for these exceptions.

Concentration of Carotenoids and Vitamin A in Blood Serum

Total carotenoids and vitamin A were measured in the serum of venous blood.   Since the cows were on a high carotenoid intake during the early stages of the experiment, the non-saponification method of Boyer et al. (3) was chosen in lieu of the more generally used Kimble (16) procedure, which is recognized to be inaccurate for vitamin A measurements in the presence of high concentrations of carotenoids (15).   Though the non-saponification method of Boyer et al. (3) apparently is unsuitable for dog blood, which is presumed to be high in the ester form of vitamin A, this procedure was reported to be applicable to normal bovine blood.

TABLE 2

Average concentrations of vitamin A and carotenoids in the blood serum of cows on different levels of vitamin A intake

Daily supplement of vitamin A	Breed of cows	No. of cows	Vitamin A and carotenoids in serum*			
			Dec. 11–26, 1944		Jan. 22–26, 1945	
			Vitamin A	Carotenoids	Vitamin A	Carotenoids
			$\gamma/100ml.$	$\gamma/100ml.$	$\gamma/100ml.$	$\gamma/100ml.$
None	Guernsey	2	23.2	1007	22.8	487
	Holstein	5	20.2	617	18.6	385
	Ayrshire	2	8.7†	279†	21.6	340
1,250,000 USP units	Guernsey	2	23.5	501	27.1	239
	Holstein	5	26.0	310	24.6	177
	Ayrshire	2	23.6†	234†	33.9	245

* Boyer et al. (3) non-saponification procedure.
† One week postpartum.

Effect of the diet on the concentration of vitamin A and carotenoids in the blood serum.   The carotenoid and the vitamin A values in table 2 are averages of assays of blood serum samples collected from individual cows of the three breeds in the respective groups.   The first period of collection, a span of 14 days, was 6 weeks after the initiation of the trial and approximately 1 month after discontinuing rye pasture; the second period of 7 days was 1 month later, near the termination of the trial.

The vitamin A content of the blood serum of the supplemented cows was higher than in the controls, but the carotenoid values were lower.   The magnitude of the difference in vitamin A concentration tended to vary with breeds, being least in the Guernsey and greatest in the Ayrshire.

The concentration of carotenoids in the serum from the Guernsey and the Holstein breeds decreased from the first period to the second, but the vitamin A values showed no significant changes.   This marked decline of the carotenoids probably was due to the continued reduction of reserves fol-

lowing removal from rye pasture and to a decrease in the carotene content
of the hay consumed. Though the Ayrshires were subjected to the same
dietary regime as the other two breeds, the vitamin A was low during the
first period as a result of a reduction associated with parturition (5, 17, 22).
With postpartum physiological readjustments, the vitamin A concentration
increased to a decidedly higher level, whereas the carotenoids changed very
little.

Relation of the analytical procedure to vitamin A values of serum. Since
the differences in vitamin A concentration in the serum from cows of the
respective groups were not of the magnitude observed in similar experiments
by other investigators (9, 15), the non-saponification procedure of Boyer
et al. (3), by which the values in table 2 were obtained, was compared with

TABLE 3

*Comparison of methods of determining vitamin A in the serum of cows on
different levels of vitamin A intake*

Daily supplement of vitamin A	Breed of cows	No. of cows	Vitamin A values by different methods		
			Kimble	Boyer *et al.**	Difference
			γ/100ml.	γ/100ml.	γ/100ml.
None	Guernsey	2	22.6	21.5	1.1
	Holstein	1	20.7	18.7	2.0
	Ayrshire	2	20.5	18.1	2.4
	Av.	5	21.4	19.6	1.8
1,250,000 USP units	Guernsey	2	29.7	23.7	6.0
	Holstein	1	39.6	30.5	9.1
	Ayrshire	2	45.1	32.9	12.2
	Av.	5	37.8	28.8	9.0

* Non-saponification method.

the Kimble (16) method. The comparison was made near the termination
of the trial when the carotenoid content of the blood serum was sufficiently
low to minimize interference.

The Kimble (16) procedure yielded higher values throughout than did
the non-saponification method of Boyer *et al.* (3), but the average difference
was greater in the vitamin A supplemented group, 31.3 per cent, than in the
non-supplemented group, 9.2 per cent (table 3). Further comparisons of
the results revealed that the average values for the supplemented cows were
76.6 per cent higher by Kimble but only 46.9 per cent higher by Boyer *et al.*
The lower values by the non-saponification method of Boyer *et al.*, particu-
larly in serum from cows receiving dietary vitamin A, suggested that either
this procedure failed to include all the vitamin A or the Kimble method
yielded excessively high values. Recent observations (19) indicate that as
the vitamin A content of serum increases from vitamin A feeding, the values
obtained by the non-saponification procedure of Boyer *et al.* tend to be too
low. In view of this, it is probable that the total vitamin A in the serum of

the cows receiving the vitamin A supplemented ration was nearer the level indicated by the Kimble method than that by the Boyer *et al.* This phase of the problem is being investigated further.
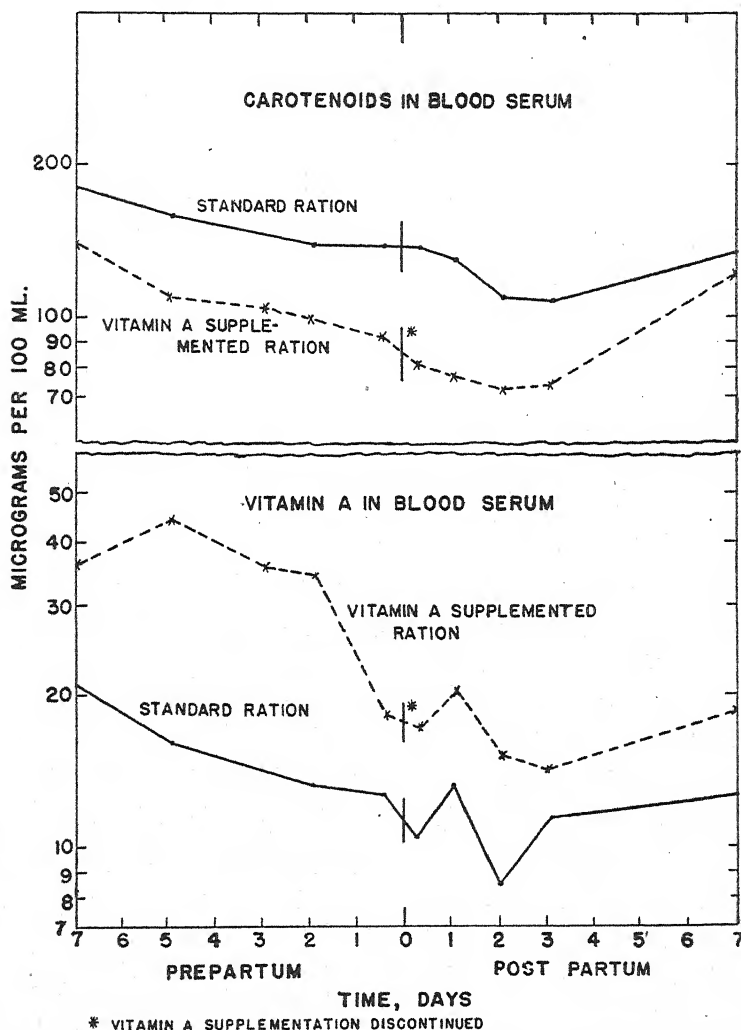
FIG. 2. The effect of prepartal dietary supplementation of vitamin A on the carotenoid and the vitamin A concentration in the blood serum of the dairy cow during the stages of terminal gestation and initial lactation.

A preferable method would have been the saponification procedure outlined by Boyer *et al.* (3), but early attempts to apply it yielded spurious results. Subsequent investigation revealed that the difficulty was due to a contaminant, presumably aldehyde (12), in the alcohol used.

Effect of prepartal vitamin A intake on the changes of carotenoids and

vitamin A in the serum of the parturient cow. As suggested by the average vitamin A values in the serum from cows of the Ayrshire breed (table 2), the vitamin A supplementation did not prevent the usual gestational reduction but did maintain a higher level than observed in the control cows. This is illustrated further (fig. 2) by the prepartum and the postpartum changes in the blood serum of an Ayrshire cow from each of the two dietary groups, control and vitamin A supplemented. Though the two cows selected had about the same carotenoid and vitamin A levels prior to supplementation, dietary vitamin A increased the vitamin A of the blood serum but reduced the carotenoids, as measured by the Kimble method (16) of analysis. The curves, on a semi-logarithmic scale, show that in both animals the prepartum rate of vitamin A decline was more pronounced than that of the carotenoids. The drop of vitamin A, however, was more precipitous in the serum of the cow receiving vitamin A. A temporary rise in the concentration of vitamin A was noted in both cows the day following parturition. Further data (19) indicate that this phenomenon also is common in other cows, but the frequency of occurrence is unknown and the factors involved are obscure. The minimum postpartum concentration usually occurred about the third day unless complicated by infections (5). Whether or not continued postpartum supplementation would have accelerated the rate of recovery when adequate liver stores were available is problematical.

Concentration of Vitamin A in the Livers and in the Serum of Cows Slaughtered

Livers were salvaged from seven cows to determine the effect of vitamin A intake on storage. During the week prior to slaughtering, blood samples were collected for vitamin A determinations. The assay procedure for livers was a slight modification (25) of the Guilbert and Hart (13) method, and for blood serum the non-saponification procedure of Boyer *et al.* (3) was used. With the exception of one animal, no. 169, vitamin A supplementation was continued to within 24 hours of slaughtering.

When an abundance of vitamin A was present in the daily ration, the cows accumulated pronounced liver reserves of this vitamin, approximately four times the amount detected in the livers of cows on unfortified rations (table 4). Though several of the livers had isolated abscesses, this pathological condition apparently did not interfere with vitamin A storage. If it is assumed that prior to cessation of supplementation the liver reserves of cow no. 169 had reached the same general level as in the other cows of her group, the rate of depletion of vitamin A stores was rapid. The vitamin A concentration in the serum from the individual cows revealed, in accord with the report of Braun (6), no correlation between the liver reserves and the levels in the blood serum.

TABLE 4

Concentrations of vitamin A in the livers and in the serum of cows on different levels of vitamin A intake

Daily supplement of vitamin A	Breed of cows	Herd no.	Wt. of liver	Vitamin A		Remarks
				Liver	Serum	
			kg.	γ/g.*	γ/100 ml.	
None	Holstein	104A	8.2	150	26.4	Non-breeder. Healthy liver.
	Ayrshire	222A	7.5	142	23.4	Brucellosis reactor. Healthy liver.
	Jersey	332A	5.2	138	19.3	Brucellosis reactor. Healthy liver.
1,250,000 USP units	Holstein	144	9.5	600	28.8	Non-breeder. Mastitic. Abscesses in liver.
	Holstein	161	8.6	733	23.8	Mastitic. Healthy liver.
	Holstein	169	8.9	421	27.2	Vitamin A withheld 20 days preceding slaughter. Mastitic. Abscesses in liver.
	Holstein	173	9.8	867	23.2	Mastitic. Malignant growths in carcass.

* Wet basis.

DISCUSSION

The data presented on milk and milk fat confirm reports by other investigators (2, 10, 15, 18, 21) indicating that production is not stimulated by vitamin A supplementation when the lactating cows are in a good state of nutrition. Probably when favorable responses are elicited by vitamin A feeding (1, 7, 8, 23, 24) either this vitamin *per se* or some other nutrient for which an increased level of vitamin A tends to compensate is the limiting factor. The apparent adequacy of rations for lactating cows at any particular time may be misleading unless cognizance is taken of their nutritional history, productive capacity, and feed consumption. Wilson (24) suggested that access to good quality roughages high in carotene does not insure adequate intake, particularly by high producing cows that have much of their feed capacity utilized by rations low in vitamin A active substances. It is conceivable that in many herds a slight submarginal deficiency of vitamin A may prevail as a result of unrecognized depletion. In these cases a favorable production response to vitamin A feeding would be expected.

Since "dry vitamin A" supplementation did not depress the milk fat percentage, as is observed commonly when cod-liver oil is fed, it is probable that the unsaturated fatty acids that are believed to cause the toxic reaction were not present in sufficient amounts to affect the mammary function. This indicates that the vitamin A concentrate used in this investigation may be

fed in sufficient quantities to increase the vitamin A potency of the milk without adversely affecting the fat content.

The observed increases in the vitamin A potency of the milk from dietary supplementation are in accord with the findings of others (1, 2, 7, 8, 9, 10, 15, 18, 21, 24). This means of fortifying milk, however, is uneconomical since the efficiency of secretion of ingested vitamin A is exceptionally low (8, 9, 15). Moreover, the concomitant reduction of carotene with increases in vitamin A (2, 8, 9, 10, 15) suggests that dietary vitamin A possibly reduces the nutritional value of carotenoids in the ration, thus presenting a provocative problem.

The interference of carotenoid metabolism has been ascribed to vitamin A *per se* rather than to other associated constituents (9, 20). Several possible explanations of this phenomenon have been presented. According to Hickman (14), " *in vitro* experiments show that vitamin A is a specific prooxidant for beta-carotene, lycopene, and probably zeaxanthin." Data supplementary to those already presented showed that the carotenoid concentration in a composite sample of feces from cows on a standard ration was approximately the same as in a similar sample from vitamin A supplemented cows. The vitamin A content of the feces from the latter group, however, was about 60 per cent higher. Either vitamin A was not a factor affecting the carotenoids in the bowel or it simultaneously suppressed absorption and accelerated oxidation. Recent studies (20) with chickens revealed retarded pigmentation of the shanks after cessation of vitamin A supplementation. Deuel *et al.* (9) noted a similar post-supplementation lag in recovery of carotenoid levels in dairy cows. The foregoing observations indicate that the carotenoid suppression is not exclusively an intestinal phenomenon.

A further explanation advanced by Deuel *et al.* (9) is that increases of vitamin A accelerate the destruction of carotenoids in the tissues through the development of a new enzyme system. It was suggested also that this enzyme system may destroy vitamin A. If this proves to be correct, feeding massive amounts of vitamin A over a prolonged period may be detrimental to the organism instead of beneficial.

Another viewpoint is that vitamin A may aid in the conversion of certain carotenoids to this vitamin, thus enhancing the accumulation of a maximum reserve. If it is assumed further that the capacity for storage in the body is limited, the suggested reduction of vitamin A (20) might be an accelerated elimination after the threshold is reached instead of a process of systemic destruction.

Though a decline of carotenoids and vitamin A of the blood seems to be a normal accompaniment of parturition (17, 22), the specific causes of this depression are obscure. A drop occurs regardless of the prepartal intake, but Kuhlman and Gallup (17) observed that the percentage decrease of carotene was related directly to its level in the plasma. This, as indicated by data reported herein, seems to apply to vitamin A levels also.

Attempts to associate these changes of carotenoid and vitamin A concentrations of the blood with mammary function have yielded negative results.   Although the secretion of colostrum withdraws vast amounts of nutrients from the blood, Sutton *et al.* (22) found no statistical correlation between levels of carotene and vitamin A of plasma and the output of these constituents in colostrum.   Braun (5) reported that a temporary reduction of vitamin A occurred when cows aborted, under which conditions colostrum secretion would be negligible.   Similar reductions of carotenoids and vitamin A were observed in a mammectomized cow following premature calving (26).   As suggested by Sutton *et al.* (22), many factors and complex interrelationships may be involved.   Investigation of the endocrinological aspects of the problems may aid in clarification.

The regulatory role of the liver in maintaining vitamin A concentrations in the blood, particularly in advanced gestation and early lactation, has not been elucidated.   The reserves in this organ apparently are not a limiting factor except at subnormal storage levels.   The changes in the vitamin A concentrations of the liver during this critical transitory period in the reproductive cycle merit study.

The amount of vitamin A in the livers of cows can be modified, as noted by others (6, 13), by dietary means.   Data presented by Braun (6) suggested an optimum level for storage in this organ, but a comparison of his results with those reported herein indicates a wide margin between the optimum and the possible maximum levels attainable.   Though the concentrations in the livers of the supplemented cows were at a uniformly high level, this does not indicate that the maximum was attained.   Present information on the subject raises the question of whether or not the maximum attainable concentration of vitamin A in the liver is the same from carotenoid feeding as from vitamin A supplementation.

Most nutritional studies with dairy cattle have been directed toward determinations of effects of deficiencies and the establishment of minimum requirements.   The results of this study suggest the need for considering the results from optimum and/or excess quantities of nutrients in the diet.

SUMMARY

The effects of daily supplementary feeding of 1,250,000 USP units of vitamin A in the form of a dry concentrate ("dry vitamin A") to individual lactating cows over a period of 3 months were compared with the results from similar cows on a standard dietary regime.   The following conclusions were reached:

1. Vitamin A feeding had no significant effect on total milk and fat production.

2. The "dry vitamin A" concentrate did not depress the milk fat percentage.

3. The high intake of vitamin A increased the concentration of this vitamin markedly in the milk fat but tended to suppress the carotenoid content.

4. Prolonged dietary supplementation of vitamin A increased the level of this vitamin in the blood serum but reduced the carotenoid values. The apparent magnitude of the vitamin A values varied with the analytical procedure used in the assay.

5. Supplemental feeding of vitamin A throughout the terminal stages of gestation did not prevent the characteristic declines at parturition but did maintain a higher level at this period than observed in non-supplemented cows.

6. The differences in vitamin A intake were reflected in the concentrations of vitamin A in the liver. There was no evidence of a correlation between vitamin A levels in the blood and in the liver of any of the cows that were slaughtered.

REFERENCES

(1) ARCHIBALD, J. G., AND PARSONS, C. H. The Effect of Feeding Vitamin A Supplement to Dairy Cattle. Mass. Agr. Expt. Sta. Bul. 357. 1939.

(2) BLAXTER, K. L., KON, S. K., AND THOMPSON, S. Y. The Effect of Feeding Shark-liver Oil to Cows on the Yield and Composition, and on the Vitamin A and Carotene Content of the Milk. Jour. Dairy Res., 14(3): 225–230. 1946.

(3) BOYER, P. D., PHILLIPS, P. H., AND SMITH, J. K. The Separation of Carotene from Vitamin A for the Determination of Vitamin A in the Blood Plasma. Jour. Biol. Chem., 152(2): 445–452. 1944.

(4) BOYER, P. D., SPITZER, R., JENSEN, C., AND PHILLIPS, P. H. Determination of Vitamin A and Carotene in Milk. A Rapid Extraction Procedure. Indus. and Engin. Chem., Analyt. Ed., 16(2): 101–102. 1944.

(5) BRAUN, W. Studies on the Carotenoid and Vitamin A Levels in Cattle. I. Seasonal Changes of the Carotenoid and Vitamin A Levels and the Normal Carotenoid-Vitamin A Ratio of the Blood. Jour. Nutr., 29(1): 61–71. 1945.

(6) BRAUN, W. Studies on the Carotenoid and Vitamin A Levels in Cattle. II. Carotenoids and Vitamin A in the Liver, Their Ratio and Their Relationship to Blood Levels. Jour. Nutr., 29(1): 72–79. 1945.

(7) DEUEL, H. J., JR., HALLIDAY, N., HALLMAN, L. F., JOHNSTON, C., AND MILLER, A. J. The Effect of Vitamin A Intake on Vitamin A Content of Butterfat. Jour. Biol. Chem., 139(1): 479–480. 1941.

(8) DEUEL, H. J., JR., HALLIDAY, N., HALLMAN, L. F., JOHNSTON, C., AND MILLER, A. J. The Production of High Vitamin A Milk by Diet. Jour. Nutr., 22(3): 303–313. 1941.

(9) DEUEL, H. J., JR., HALLMAN, L. F., JOHNSTON, C., AND MATTSON, F. The Effect of a High Vitamin A Intake on the Blood and Milk Carotene of Holstein and Guernsey Cows. Jour. Nutr., 23(6): 567–579. 1942.

(10) FOUNTAINE, F. C., AND BOLIN, D. W.   The Milk and Butterfat Production Responses
        to Shark Liver Oil in the Ration.   Jour. Dairy Sci., 27(2) : 155–158.   1944.

(11) GAINES, W. L.   The Energy Basis of Measuring Milk Yield in Dairy Cows.   Ill.
        Agr. Expt. Sta. Bul. 308.   1928.

(12) GALLUP, W. D., AND HOEFER, J. A.   Determination of Vitamin A in Liver.   Indus.
        and Engin. Chem., Analyt. Ed., 18(5) : 288–290.   1946.

(13) GUILBERT, H. R., AND HART, G. H.   Storage of Vitamin A in Cattle.   Jour. Nutr.,
        8(1) : 25–44.   1934.

(14) HICKMAN, K.   Quotation from a personal communication cited in Science,
        103(2680) : 585.   1946.

(15) JENSEN, C., BOYER, P. D., PHILLIPS, P. H., RUPEL, I. W., AND LUNDQUIST, N. S.
        The Effect of Shark Liver Oil on Milk and Butter Fat Production.   Jour. Dairy
        Sci., 25(11) : 931–937.   1942.

(16) KIMBLE, M. S.   The Photoelectric Determination of Vitamin A and Carotene in
        Human Plasma.   Jour. Lab. and Clin. Med., 24(10) : 1055–1065.   1939.

(17) KUHLMAN, A. H., AND GALLUP, W. D.   Changes in Blood-plasma Carotene Asso-
        ciated with Parturition and Lactation of Jersey Cows.   Jour. Dairy Sci., 27(8) :
        633–634.   1944.

(18) LUCAS, H. L., LOOSLI, J. K., AND MAYNARD, L. A.   A Study of the Effect of Dietary
        Fat and Fat-soluble Vitamins upon Milk and Fat Secretion.   N. Y. (Cornell)
        Agr. Expt. Sta. Mem. 251.   1943.

(19) PARRISH, D. B., WISE, G. H., AND HUGHES, J. S.   Unpublished Data.   Kans. Agr.
        Agr. Expt. Sta.   1946.

(20) RUBIN, M., AND BIRD, H. R.   The Apparent Antagonism between Vitamin A and
        Carotenoids in the Fowl.   Science, 103(2680) : 584–586.   1946.

(21) RUSOFF, L. L., SKIPPER, H. E., AND DIX ARNOLD, P. T.   Shark Liver Oil and the
        Vitamin A Potency of Milk.   Jour. Dairy Sci., 25(9) : 807–813.   1942.

(22) SUTTON, T. S., KAESER, H. E., AND SOLDNER, P. A.   Changes in the Level of Vita-
        min A and Carotene in the Plasma of Dairy Cows Associated with Parturition
        and Beginning Lactation.   Jour. Dairy Sci., 28(12) : 933–939.   1945.

(23) WILSON, L. T.   Feeding Vitamin A to Dairy Cows to Increase Milk Production.
        Certified Milk, 17(197) : 5.   1942.

(24) WILSON, L. T.   Vitamins for More and Better Milk.   Certified Milk, 19(223) : 3.
        1944.

(25) WISE, G. H., CALDWELL, M. J., AND HUGHES, J. S.   The Effect of the Prepartum
        Diet of the Cow on the Vitamin A Reserves of Her Newborn Offspring.   Science,
        103(2681) : 616–618.   1946.

(26) WISE, G. H., PARRISH, D. B., FRANK, E. R., AND LEASURE, L. L.   Unpublished Data.
        Kans. Agr. Expt. Sta.   1946.

COBALT IN COWS' MILK[1]

J. G. ARCHIBALD

Massachusetts Agricultural Experiment Station, Amherst

In continuation of a comprehensive project on the minerals of cows' milk, three reports on which have been published (1, 2, 3), the effect of feeding cobaltous acetate on the cobalt content of milk has been investigated. The feeding trials were conducted during the winter of 1943–44, but because of difficulty in finding a sufficiently sensitive method for the determination of extremely minute amounts of cobalt, the analytical work has been completed only recently.

Comparatively little information is recorded regarding the cobalt content of milk. Probably because of the difficulties of analysis, some of the earlier investigators (5, 7, 8, 13, 14) either do not report cobalt as a constituent of milk or else claim it to be absent, although one pair of workers (7) notes that "the complete absence of cobalt is somewhat unexpected, for it is an element with active biological properties." In 1933, Stare and Elvehjem (10) concluded that the cobalt content of milk is less than 0.01 mg. per 100 g. (100 μg. per liter of milk). By 1938, methods had been sufficiently refined so that Underwood and Elvehjem (12) report a range of 8–18 μg. per liter with an average of 11 μg. The most recent value noted is that given by Ellis and Thompson (9) in 1945, who report 0.64 μg. per liter. Other values recorded range from 1 μg. per liter (11) to 10–15 μg. per liter (5).

Data relative to the influence of the amount of cobalt in the feed on the cobalt content of milk are limited. In 1942, Askew (4) reported that cows having access to cobaltized salt licks produced milk containing 0.020–0.022 p.p.m. (20–22 μg./l.) of cobalt, as compared to 0.010–0.015 p.p.m. (10–15 μg./l.) in the milk of control animals. In the light of recent refinements of method, these values are probably too high, but the relative relationship is of interest. Comar *et al.* (6) noted a very small, unspecified amount of cobalt in milk when the radioactive element was introduced into the blood stream of cows. However, when it was introduced directly into the rumen, cobalt was not detected in the milk.

EXPERIMENTAL

The procedure was similar to that described in an earlier paper (1). Eight cows were divided into two groups of four each, each group consisting of an Ayrshire, a Guernsey, a Holstein, and a milking Shorthorn. Three of the breed pairs were matched with respect to stage of lactation, none being beyond the 12th week in lactation when the trial was started. The fourth

pair (the Shorthorns) were in the 8th and 18th week of lactation, respectively, at the start, a matched pair not being available. One group received the supplement during December and January; the other group received it during February and March. The amount of cobaltous acetate fed was 500 mg. daily or the approximate equivalent of 120 mg. of elemental cobalt. Except for the feeding of the supplement, the rations and management of the two groups were identical.

Composite 2-day milk samples of two liters each were taken from each cow once a month. Cobalt was determined in triplicate on 500-ml. portions of each sample by the method of Ellis and Thompson (9), using the alternate carbamate extraction procedure. This method, although requiring much preliminary procedure in the purification of reagents, extreme precautions in technique and the scrupulous cleaning of glassware in order to avoid contamination, proved to be very satisfactory. The standard curve for cobalt established at the start was checked twice throughout the course of the work and was found to give good agreement; recovery of added amounts of cobalt ranged from 97 to 104 per cent of theory. A Model 11 Coleman spectrophotometer was used at a wave length of 345 millimicrons with filter PC6 and absorption tubes 50 mm. long and 10 mm. in diameter, with a capacity of approximately 3 ml. Final volume of the unknown solution was usually between 4 and 5 ml. A continuous glass still for production of double distilled water is a prerequisite for this type of work.

Previous to adoption of this method an older method which makes use of nitroso-R salt as the specific reagent for cobalt was given extensive trial, but proved quite unsuited to the purpose. Spectrographic analysis also was tried but was abandoned when it failed to reveal the presence of cobalt in dilutions of the magnitude anticipated. At already noted, other investigators (5, 13) also have been unable to identify cobalt in milk ash by means of the spectrograph.

RESULTS

The values obtained are summarized in table 1. The amount of cobalt occurring naturally in these milks averaged about 0.6 μg. per liter, with a range from 0.2 to 1.14 μg. These values are of the same order of magnitude as those reported by Ellis and Thompson (9) and Sylvester and Lampitt (11). There was some tendency for the amount of cobalt in the control milks to diminish as the season advanced. A similar tendency was noted in earlier work on manganese (1). The question raised at that time regarding that element appropriately may be raised with respect to cobalt, *viz.*: Does this mean that possibly, during the pasture season, cows store a reserve of the element which tends to become depleted as the winter season on dry feed proceeds?

Without exception, the milk from a cow receiving supplemental cobalt was higher in the element than the milk from her breed mate not receiving

TABLE 1

Effect on cobalt content of the milk of feeding cows cobaltous acetate

(Micrograms of cobalt per liter of milk)

Cows on control ration

1st half of the season

Month	*A327	G658	H581	S62	Average of all four
December	1.0	1.0	0.6	0.9	0.9
January	0.8	0.7	0.5	0.3	0.6
Av. 1st half	0.9	0.9	0.6	0.6	0.7

2nd half of the season

Month	A298	G640	H567	S38	Average of all four
February	0.9	1.1	0.5	0.4	0.7
March	0.2	0.2	0.2	0.2	0.2
Av. 2nd half	0.6	0.7	0.4	0.3	0.5
Av. entire season	0.7	0.8	0.5	0.4	0.6

Cows receiving supplemental cobalt

1st half of the season

Month	A298	G640	H567	S38	Average of all four
December	2.3	1.6	1.0	1.5	1.6
January	2.6	2.9	1.4	1.8	2.2
Av. 1st half	2.5	2.3	1.2	1.6	1.9

2nd half of the season

Month	A327	G658	H581	S62	Average of all four
February	2.5	2.3	1.4	5.9†	3.0
March	2.7	2.9	0.9	5.0†	2.9
Av. 2nd half	2.6	2.6	1.2	5.4	2.9
Av. entire season	2.6	2.4	1.2	3.5	2.4

* The initial letter prefixed to each cow's number indicates the breed.

† Although there is nothing in the history of these two samples that would lead one to suspect contamination, they are so much out of line with the other values obtained that their validity may be open to question. However, since a high value for this cow was obtained in two successive months, the results are included in the average.

it.   The increase ranged from less than two-fold (1.7—Holsteins—December) to twenty-five-fold (Shorthorns—March).   As noted in the table, the latter value may be questioned.   Of more interest is the average increase which was four-fold, a value which is highly significant statistically.

Although some variations between breeds are evident, the only consistent differences are the relatively low values for cobalt in Holstein milk, both when the cows were on the control ration and when they were receiving the supplement.

The obvious possible significance of these results lies in their application to calf nutrition.   In our experience, young stock have shown greater susceptibility to the nutritional anemia which is characteristic of cobalt deficiency than have older cattle.   In the light of these results it would seem that in areas where cobalt deficiency is common, the requirements of calves for this element might most naturally and logically be supplied through the milk of cows whose rations have been fortified with supplemental cobalt. Since many of our feed manufacturers now include cobalt regularly in the formulation of ready mixed rations for dairy cows, the use of such rations would tend to automatically supply the needs of the young calf for cobalt. Under ordinary circumstances the calf's requirements for cobalt may not be met since some of the control milks in this investigation contained less cobalt than was found in the drinking water supplied to the cows (0.21 μg./l. as compared with 0.26 μg./l.).

SUMMARY

Cobaltous acetate was fed as a supplement (500 mg. daily) to the rations of eight cows for a period of 2 months by the double reversal method and the milk was analyzed for cobalt.   The results revealed that feeding the supplement consistently raised the amount of cobalt in the milk.   The average increase was four-fold.   The milk from cows receiving the supplement averaged 2.4 μg. of cobalt per liter in contrast with 0.6 μg. per liter when the cows were on the control ration.   The possible significance of the results is discussed briefly.

REFERENCES

(1) ARCHIBALD, J. G.   Manganese in Cows' Milk.   Milk Plant Monthly, 30(9): 36. 1941.

(2) ARCHIBALD, J. G.   Zinc in Cows' Milk.   Jour. Dairy Sci., 27: 257–261.   1944.

(3) ARCHIBALD, J. G., AND LINDQUIST, H. G.   Manganese in Cows' Milk.   Jour. Dairy Sci., 26: 325–330.   1943.

(4) ASKEW, H. O. Mineral Content of Pastures. Investigations at the Cawthron Institute. New Zeal. Dept. Sci. and Indus. Res., Ann. Rpt., 16: 13–15. 1942.

(5) BLUMBERG, H., AND RASK, O. S. The Spectrographic Analysis of Milk Ashes. Jour. Nutr., 6: 285–288. 1933.

(6) COMAR, C. L., DAVIS, G. K., TAYLOR, R. F., HUFFMAN, C. F., AND ELY, R. E. Cobalt Metabolism Studies. II. Partition of Radioactive Cobalt by a Rumen Fistula Cow. Jour. Nutr., 32: 61–68. 1946.

(7) DINGLE, H., AND SHELDON, J. H. A Spectrographic Examination of the Mineral Content of Human and Other Milk. Biochem. Jour., 32: 1078–1086. 1938.

(8) DREA, W. F. Spectrum Analysis of Milk Ashes. Jour. Nutr., 8: 229–234. 1934; also, 16: 325–331. 1938.

(9) ELLIS, G. H., AND THOMPSON, J. F. Determination of Cobalt in Biological Materials with Nitrosocresol. Indus. and Engin. Chem., Analyt. Ed., 17: 254–257. 1945.

(10) STARE, F. J., AND ELVEHJEM, C. A. Cobalt in Animal Nutrition. Jour. Biol. Chem., 99: 473–483. 1933.

(11) SYLVESTER, N. D., AND LAMPITT, L. H. The Determination of Cobalt in Foods. Soc. Chem. Indus. Jour., Trans. and Commun., 59: 57–60. 1940.

(12) UNDERWOOD, E. J., AND ELVEHJEM, C. A. Is Cobalt of Any Significance in the Treatment of Milk Anemia with Iron and Copper? Jour. Biol. Chem., 124: 419–424. 1938.

(13) WRIGHT, N. C., AND PAPISH, J. The Inorganic Constituents of Milk. Science, 69: 78. 1929.

(14) ZBINDEN, C. The Infinitely Small Quantities of Certain Elements in Milk and Their Detection by the Spectrographic Method. Lait, 11: 114. 1931.

THE NUTRITIVE VALUE OF FRACTIONS OF BUTTERFAT PREPARED BY COLD CRYSTALLIZATION[1]

R. P. GEYER, B. R. GEYER, P. H. DERSE, H. NATH,[2] V. H. BARKI,
C. A. ELVEHJEM, AND E. B. HART

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

In past work the following methods have been employed to concentrate and characterize the factor(s) in butterfat responsible for its superior nutritive value as compared with corn oil: lead soap separation and steam distillation of butterfat fatty acids (2, 10), fractional distillation of the methyl esters of butterfat fatty acids (5), and chromatographic procedures (3). Since many of these require conditions conducive to chemical changes, cold crystallization of an acetone solution of butterfat was selected as a procedure most likely to yield fractions which had undergone little chemical transformation.

Henry *et al.* (7) reported the nutritive value of three fractions of butterfat prepared by cold crystallization from an acetone solution of 0° C. Two of these products with iodine numbers of 19 and 47 were fed to rats in a skimmed milk ration. The rate of growth was best on the diet containing the liquid fraction, but the authors stated that the small differences in gains between groups were without statistical significance. In 1946 Jack *et al.* (8) published the results on the nutritive value of five different fractions of butterfat prepared by low-temperature crystallization from hexane. Successive temperatures of $-7, -13, -23,$ and $-53°$ C. were used, and in each case the precipitate was removed. The final filtrate was concentrated and yielded the fifth fraction. To test the effect of solvent treatment on the nutritive value of the fat, butterfat was dissolved in hexane and the solvent removed by distillation. The fractions as well as the treated and untreated butterfat were incorporated into a synthetic type ration and fed to groups of rats. The poorest gain was made by the animals receiving the $-7°$ C. precipitate (m.p. 53° C.) and the best gain was made by the animals fed either the untreated butterfat or the $-53°$ C. filtrate rations. The solvent-treated butterfat proved to be definitely inferior to the untreated fat.

The experiments described in the present paper concern the application of cold crystallization to samples of butterfat obtained at various times throughout the year. The nutritive value and some chemical and physical constants of the fractions prepared are reported.

EXPERIMENTAL

The butterfat used in these experiments was prepared by decantation and filtration of melted (65° C.) unsalted sweet cream butter obtained from the University Creamery. The acetone was redistilled in an all glass still from calcium chloride before being used. One kilogram of melted butterfat was dissolved in 10 liters of acetone, and after being thoroughly stirred, the solution was cooled to −4° C. and kept at this temperature for 24 hours. Filtration of the yellow supernatant liquid from the white granular precipitate was accomplished by means of a submerged pressure filter. The precipitate was recrystallized twice from 5 liters of acetone at −4° C. The filtrates were combined and concentrated under partial vacuum, using a nitrogen ebullition tube. The temperature was kept below 20° C. except during the removal of final traces of solvent, when more heat was applied and a vigorous stream of nitrogen was used. The residue was reinforced with the proper amounts of the fat-soluble vitamins and then was stored at −6° C. This preparation was designated as butterfat fraction 2 (BF–2). The solid fraction remaining after the recrystallizations was freed of solvent and was vitaminized and stored at −6° C. This product was designated as butterfat fraction 1 (BF–1). The butterfat used in Expt. 1 was obtained in September, 1945; the fractions made at that time were stored for a number of weeks before use. Melting points were determined by a modified Wiley method and iodine numbers by the Hanus method. To eliminate the possible effect that the acetone treatment might have on the nutritive value of the fat fractions, a sample of butterfat was dissolved in acetone and after several hours the solvent was removed as described above.

Corn oil[3] was separated into two fractions in the following manner: 1 kg. of corn oil was dissolved in 5 liters of Skellysolve A and the mixture was cooled to −45° C. by use of an acetone–dry ice bath. After 5 hours at this temperature filtration was accomplished by means of the submerged filter. Because of the "waxy" nature of the precipitate some difficulty was encountered at this stage. Two recrystallizations of the solid material were made from 2,500-ml. portions of solvent at −45° C. The filtrates were combined, concentrated and vitaminized as previously described, and were designated corn oil fraction 2 (CO–2). Similar treatment of the solid product yielded corn oil fraction 1 (CO–1). A sample of corn oil was dissolved in the solvent and then freed of it after several hours to give a solvent-treated corn oil (CO–S).

[3] Mazola brand. Corn Products Refining Co.

Groups of six male weanling rats of the Sprague-Dawley strain weighing 40–45 g. were placed on rations containing each of the following fats: butterfat (BF), corn oil (CO), solvent-treated butterfat (BF–S), solvent-treated corn oil (CO–S), and the fractions BF–1, BF–2, CO–1, or CO–2. The basal ration consisted of:

Casein (alcohol extracted)[4]	20%
Fat	28%
Sucrose	48%
Salts IV (7)	4%
Vitamins per 100 g. of ration:	
Thiamine	200 gamma
Riboflavin	300 gamma
Pyridoxine	300 gamma
Ca pantothenate	1500 gamma
Choline hydrochloride	100 mg.
β-Carotene[5]	560 gamma
α-Tocopherol	2240 gamma
Calciferol[6]	14 gamma
2-methyl-1,4-naphthoquinone	210 gamma

Food consumption was unrestricted. Weekly weight records were kept over a 6-week period, and the results are given in table 1. Because of a marked difference in the appearance of the feces of the rats fed the two fat fractions, feces were collected from each group and analyzed for total fat, ash, and bound fatty acids. The latter determination was carried out by warming the ether-extracted feces with dilute HCl (1 : 4) for 30 minutes and extracting again with ether. The following results were obtained:

Group	Description of feces			
	Color	% Ash	% Neutral fat	% Bound fatty acids
BF–1	White	15.8	7.8	66.7
BF–2	Black	24.7	15.5	32.4
BF	Mixed	17.8	11.0	50.8

Because of the rapid rate of growth of the animals fed the BF–2 ration, studies were undertaken to investigate this phenomenon further. Fractions similar to those used in Expt. 1 were prepared using butterfat made from butter of December, 1945, and from February, June, July, and September of 1946. In all cases the butter was obtained directly after churning, and the fractionations and animal feeding trials were made without delay. In addition, butter obtained from the Quartermaster Corps of the Army and rated by them as June, 1945, also was tested. In a few instances additional

[4] Extracted for three 2-hour periods with boiling alcohol.
[5] 90 per cent β-carotene and 10 per cent α-carotene.
[6] Crystalline irradiated ergosterol.

fractions were prepared. The nutritional value of these fats and their fractions was tested by incorporating them in the basal diet used in Expt. 1 and

TABLE 1

Results of cold crystallization experiments
(Each figure represents the average of six rats)

Expt. no.	Group	Description of fat portion	m.p. °C.	Iodine no. (Hanus)	Av. gain 4 wks.	Av. gain 6 wks.
					(g.)	(g.)
1 Sept., 1945	BF*	Sept., 1945	34.0	33	101	158
	BF-S	Acetone treated	34.0	33	97	146
	BF-1	-4° C. ppt.	42.1	27	50	86
	BF-2	-4° C. filtrate	8.0	47	139	204
	CO*	Mazola	....	114	88	121
	CO-S	Skellysolve A treated	....	114	86	125
	CO-1	-45° C. ppt.	....	109	88	122
	CO-2	-45° C. filtrate	....	121	85	116
2 Dec., 1945	BF	Dec., 1945	....	....	112	163
	BF-1	-4° C. ppt.	42.5	....	90	....
	BF-2	-4° C. filtrate	7.6	....	117	171
3 Feb., 1946	BF	Feb., 1946	34.1	....	129	....
	BF-S	Acetone treated	34.0	....	123	....
	BF-1	-4° C. ppt.	41.9	....	116	....
	BF-2	-4° C. filtrate	7.5	....	124	....
	BF-1a	7° C. ppt.	47.1	11	67	....
	BF-2a	-4° C. ppt. minus fraction BF-1a	28.0	17	108	....
	BF-3a	Combined filtrates from 1a and 2a	7.2	49	113	....
4 June, 1946	BF-M	March, 1946	....	....	126	....
	BF-J	June, 1946	33.0	....	120	....
	BF-1-J	-5.5° C. ppt.	41.8	....	109	....
	BF-2-J	-5.5° C. filtrate	9.9	....	129	....
5 July, 1946	BF	July, 1946	....	....	128	189
	BF-1	-5.5° C. ppt.	....	....	104	153
	BF-2	-5.5° C. filtrate	....	....	125	186
	BF-3	-5.5° C. filtrate minus the recrystallization filtrates from BF-1			127	188
6 Sept., 1946	BF	Sept., 1946	....	....	101	173
	BF-1	-5.5° C. ppt.	....	....	111	168
	BF-2	-5.5° C. filtrate	....	....	130	197
7 June, 1945	BF-J	June, 1945, Quartermaster Corps	....	....	115	172
	BF-1-J	-5.5° C. ppt.	42.8	....	75	....
	BF-2-J	-5.5° C. filtrate	12.4	....	125	187
	BF-M	March, 1946	....	....	119	181

* BF = butterfat; CO = corn oil.

feeding each ration to a group of six male weanling rats. The results are given in table 1. Feces collections and food consumptions were made in Expts. 3, 4, and 7, and analyses were made for neutral fat and combined

TABLE 2

Feed excretion data for animals fed fats and fat fractions

(All data are based on 3-day pooled samples from six-rat groups, unless otherwise indicated)

Expt. no.	Group	Description of fat portion	Food ingested (g.)	Fat intake (g.)	Neutral fat excreted (g.)	Bound* fatty acids excreted (g.)	Lipid excreted (g.)	Fat absorbed (%)
3	BF	Feb., 1946	246	68.8	0.91	5.95	6.86	91.2
Feb., 1946	BF-1	Acetone treated	228	64.0	1.01	5.23	6.24	91.8
	BF-1	−4° C. ppt.	204	56.9	1.04	7.64	8.68	84.8
	BF-2	−4° C. filtrate	213	59.6	0.63	3.54	4.16	93.0
	BF-1a	7° C. ppt.	167	46.7	4.01	6.62	10.60	77.3
	BF-2a	−4° C. ppt. minus the 7° ppt.	176	49.2	1.03	6.09	7.11	85.8
	BF-3a	Combined filtrates	203	56.9	0.66	3.69	4.35	92.3
4†	BF-J	June, 1946	321	90.0	1.76	8.64	1.04	88.5
June, 1946	BF-J-1	−5.5° C. ppt.	329	92.0	0.94	3.86	4.81	94.8
	BF-J-2	−5.5° C. filtrate	221	62.0	0.43	3.86	4.28	93.1
	BF-M	March, 1946	342	95.9	0.97	3.56	4.53	95.3
7	BF-Q‡	June, 1945	152	42.6	1.11	3.30	4.41	89.6
	BF-Q-1	−4° C. ppt.	144	40.2	2.82	5.69	8.51	78.8
	BF-Q-2	−4° C. filtrate	198	55.5	0.50	3.57	4.07	92.7
	BF-M	March, 1946	227	63.6	0.97	4.84	5.89	90.2

* Ether extractable material liberated by HCl treatment.
† 7-day pooled samples.
‡ U. S. Army Quartermaster Corps.

fatty acids.   The latter analysis was made as described previously.   The results of these analyses are given in table 2.   The values for the per cent absorption of the various fats are not corrected for "metabolic" fat excretion.

DISCUSSION

Separation of butterfat into two fractions by means of cold crystallization from an acetone solution yielded in Expt. 1 a liquid fraction which allowed a very rapid rate of growth of rats when incorporated in a sucrose diet.   During a 6-week period these animals averaged 4.9 g. per day, a rate of growth above that usually obtained with Sprague-Dawley rats fed similar synthetic type diets (4).   The solid fraction of the butterfat proved to be very low in nutritional value, for the rats gained only an average of 2 g. per day over the 6-week period.   From the iodine number of 27 which this fraction possessed, it is evident that unsaturated acids were present; however, these acids were not identified.   It seems doubtful that linoleic acid deficiency could have caused the slow rate of growth which occurred even during the first week of the experiment.   The fecal analyses show that the rat was able to hydrolyze the fat, but that the fatty acids liberated were not well absorbed.   The melting point of 42° C. was not too high, for fairly good absorption occurred in subsequent experiments in which analogous fractions were employed.

The wide differences in nutritive value between the two fractions prepared from September, 1945, butter were not evident in any of the subsequent fractionation trials in which the butterfat used was prepared from butter obtained in June and December of 1945, and February, June, July, and September of 1946.   In most cases, however, the rats fed the butterfat fraction 1 ration grew at a rate slightly inferior to that of the control groups, and they excreted more total ether-extractable material in their feces. Growth of the rats fed the BF–2 rations was uniformly good in all experiments and the per cent absorption of this fat was always above 89 per cent. Henry et al. (7) concluded that there was no significant difference between the nutritive value of two fractions of butterfat which were obtained by a procedure similar to the one described in this paper.   The iodine numbers of their fractions are in close agreement with those given in table 1.   It should be noted that the growth of the animals used by the English workers was far below the usual growth of the animals used in the present study; also, a different ration was used.

The growth of the rats fed the two corn oil fractions was nearly identical to that of the corn oil control groups, and the gain in weight by the animals receiving either of the treated fats was equal to that of those fed the nontreated fats.   This latter finding is in disagreement with the observation of Jack et al. (8), who showed that the treatment of butterfat with hexane decreased its nutritive value.

No reason is apparent for the fact that only in the first experiment were significant differences between the nutritive value of the two fractions obtained. Perhaps a more detailed separation of butterfat will be needed to secure consistent results as far as an extremely active fraction is concerned. In view of the finding of Jansen's group of workers (1, 9) that vaccenic acid (11, 12-eladic acid) has growth-promoting properties, it would be of interest to analyze the various fractions for this compound.

SUMMARY

Butterfat prepared from butter made in various months of the year has been fractionated into two fractions by cold crystallization from an acetone solution. A liquid fraction obtained from September, 1945, butter allowed rats to grow at a superior rate, while a solid fraction prepared from this butter caused a very slow rate of growth. This phenomenon was not repeated to the same degree in subsequent trials using other samples of butter.

Corn oil was separated into two fractions by a similar procedure but rats grew equally well when fed either of these fractions or corn oil itself.

The treatment of either fat by solvent had no deleterious effect on its nutritive value.

REFERENCES

(1) BOER, J., JANSEN, B. C. P., AND KENTIE, A. Further Investigations on a Fat-soluble Growth-promoting Substance Present in Summer Butter. Nature, 158: 201. 1946.

(2) BOUTWELL, R. K., GEYER, R. P., ELVEHJEM, C. A., AND HART, E. B. The Effect of Hydrogenation on the Nutritive Value of the Fatty Acid Fractions of Butter Fat and of Certain Vegetable Oils. Jour. Dairy Sci., 24: 1027–1034. 1941.

(3) BOUTWELL, R. K., GEYER, R. P., ELVEHJEM, C. A., AND HART, E. B. Effect of Flavor on Nutritive Value of Fats. Soc. Expt. Biol. and Med. Proc., 55: 153–155. 1944.

(4) BOUTWELL, R. K., GEYER, R. P., ELVEHJEM, C. A., AND HART, E. B. Studies on the Interrelation of Fats, Carbohydrates, and B-Vitamins in Rat Nutrition. Arch. Biochem., 7: 143–157. 1945.

(5) GEYER, R. P., AND BOUTWELL, R. K. Unpublished.

(6) HEGSTED, D. M., MILLS, R. C., ELVEHJEM, C. A., AND HART, E. B. Choline in the Nutrition of Chicks. Jour. Biol. Chem., 138: 459–466. 1941.

(7) HENRY, K. M., KON, S. K., HILDITCH, T. P., AND MEARA, M. L. Comparison of the Growth-promoting Value for Rats of Butter Fat, of Margarine Fat, and of Vegetable Oils. Jour. Dairy Res., 14: 45–58. 1945.

(8) JACK, E. L., HENDERSON, J. L., REID, DELLA F., AND LEPKOVSKY, S. Nutritional Studies on Milk Fat. Jour. Nutr., 30: 175–181. 1945.

(9) KENTIE, A. Een Nieuw Vitamine in Boter. Doctorate Thesis, Universiteit von Amsterdam. 1946.

(10) SCHANTZ, E. J., BOUTWELL, R. K., ELVEHJEM, C. A., AND HART, E. B. The Nutritive Value of the Fatty Acid Fractions of Butter Fat. Jour. Dairy Sci., 23: 1205–1210. 1940.

STUDIES ON KETOSIS IN DAIRY CATTLE. IX. THERAPEUTIC EFFECT OF ADRENAL CORTICAL EXTRACTS

J. C. SHAW

Department of Dairy Industry, Storrs Agricultural Experiment Station, Storrs, Connecticut

There are some similarities between ketosis in cattle and Addison's disease in humans. Both subjects exhibit hypoglycemia and hypersensitiveness to insulin injections. The symptoms in both often are apparent at glucose levels which do not produce symptoms in normal subjects. Also in both, the blood glucose response to epinephrine injections is slight. There are a number of dissimilarities, but the positive relationships appeared to warrant some preliminary studies as to the possible rôle of the adrenal cortex in the development of ketosis in cattle.

EXPERIMENTAL

Four cases of uncomplicated ketosis were selected for study. Adrenal cortical extracts (Wilson) were injected subcutaneously at frequent intervals over short periods of time. Insofar as was possible, the cows were maintained on their customary feeding and management regime. Blood samples for glucose and acetone body determinations were drawn at frequent intervals before and during the course of treatment. Observations also were made of the behavior of the cows during the experimental periods. Blood glucose and acetone bodies were determined by methods previously cited (2).

Cow D. E. 259 was under observation for 3 days before treatment was initiated. The blood glucose, which was already at a low level on the first day of observation, decreased still more, and the blood acetone bodies increased from an already high level of 38.0 mg. per cent (fig. 1). The cow had refused all grain and ate but sparingly of hay during this period. Twenty milliliters of Wilson's extract were injected the evening of the third day. The following morning the cow appeared ravenous, quickly consuming 10 lbs. of hay and 5 lbs. of concentrate. A full bucket of grain containing approximately 10 lbs. of concentrate was fed and eaten rapidly. The blood glucose increased rapidly within the next 24 hours. Concurrently, the blood acetone bodies decreased.

The cortical extract was injected again on the fourth and fifth days, but the cow went off feed, apparently the result of over-feeding. On the seventh day, when the blood glucose and acetone bodies indicated a relapse and the appearance of the animal had worsened, injections again were initiated. Six injections, totaling 200 ml., were given within a 3-day period. The appetite of the cow again improved markedly; the blood picture likewise im-

proved.   Milk production tended to parallel the blood sugar curve rather closely, increasing during both periods of treatment (fig. 1).   The cow recovered without further treatment.

Cow D. E. 262, from which the data for figure 2 were obtained, also had shown symptoms of ketosis several days before treatment was initiated.   This animal had been off feed for several days, exhibited incoordination and
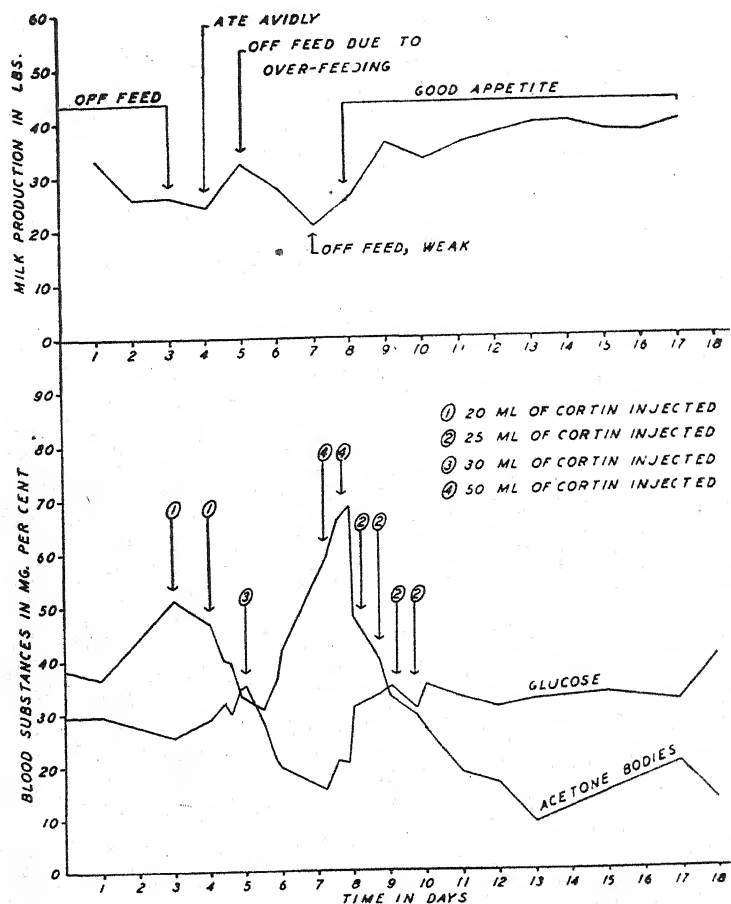
FIG. 1.   Ketosis—cortin therapy (Cow D. E. 259).

general paresis, and was becoming emaciated.   Milk production increased at first and then began to decrease rapidly, apparently with the onset of ketosis (fig. 2).

During a 3-day period, 375 ml. of Wilson's adrenal cortical extract were injected subcutaneously.   An improvement in appetite was observed within 24 hours.   The blood picture also improved, the blood glucose and acetone bodies approaching normal levels within 5 days.   Within 4 days of the last
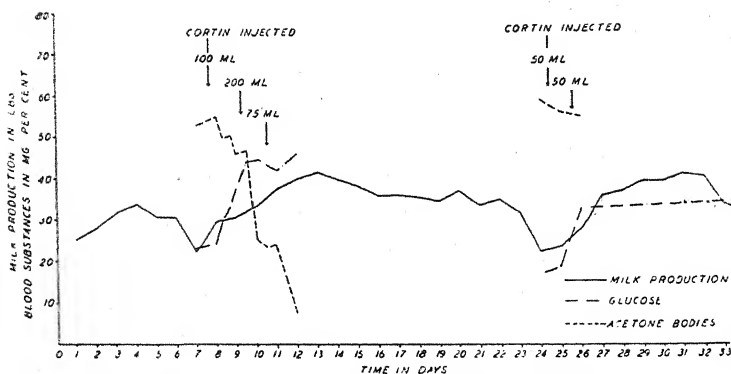
FIG. 2. Ketosis—cortin therapy (Cow D. E. 262).

injection, milk production, which had almost doubled fóllowing the initial injections, again began to decrease, indicating a relapse. Once more the animal refused all feed. An injection of 50 ml. of the adrenal cortical extract was made and repeated on the following day. Improvement in appetite, milk production, blood glucose, and blood acetone bodies was prompt. A week later, when the appearance of the animal denoted a possible relapse, glucose was injected intravenously.

In figures 3 and 4, data are presented graphically on the response to injections of adrenal cortical extracts of two cows exhibiting less advanced stages of ketosis. In both cases the ketonemia and hypoglycemia were marked, and both animals exhibited inappetence. The response to injections of the ad-
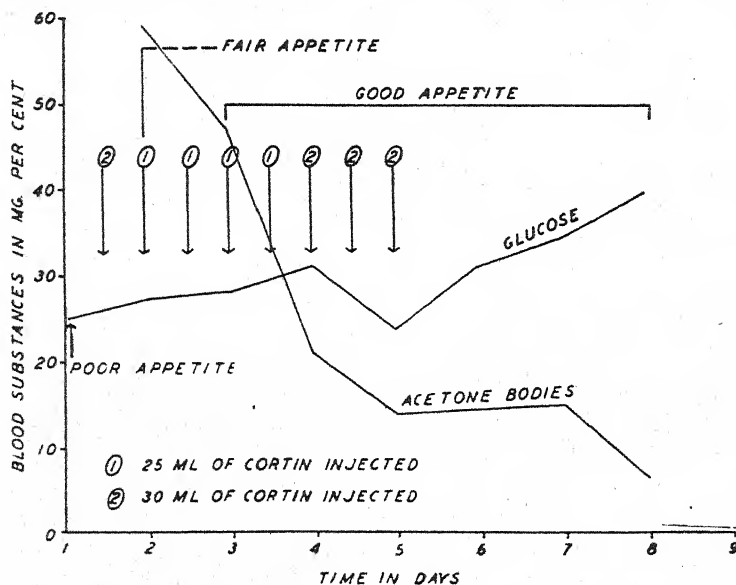
FIG. 3. Ketosis—cortin therapy (Cow D. E. 264).
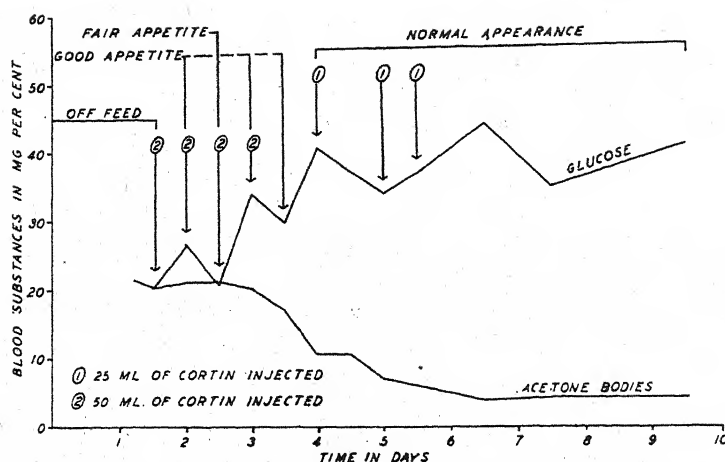
FIG. 4.   Ketosis—cortin therapy (Cow D. E. 267).

renal cortical extract was prompt in the two animals.   The appetite and general appearance of the two cows appeared normal within 3 to 4 days. No further treatment of any kind was required.

DISCUSSION

Caution must be exercised in drawing conclusions as to the effectiveness of any substance in promoting recovery of cows suffering from ketosis, since they recover from time to time without treatment and without any apparent change in management and feeding (2).   The marked improvement following each injection period in each of the four cases studied appears to be significant however.   It appears unlikely that four such cases selected at random all would improve so quickly purely by chance happening.   Rather, it appears that the adrenal cortical extract did have a specific effect in promoting recovery.

This does not necessarily mean that ketosis in cattle is due to an adrenal insufficiency, since extracts of the adrenal cortex promote glyconeogenesis even in normal animals.   The effect could have been strictly a therapeutic one in which improvement was due to an increased liver glycogen and/or blood glucose.   There appeared to be little actual measurable increase in the blood sugar until after the appetite had improved, though it is possible that more carbohydrate was being made available to the body tissues.

The following observations on cows are similar to those observed in Addison's disease (3) and favor the view that an adrenal insufficiency may be involved: (a) Ketosis in dairy cows is a hypoglycemic condition.   (b) There is a definite lowering of the glucose threshold at which symptoms become manifest (1).   (c) Adrenal cortical extracts have elicited a favorable response in each of the four cases studied.   (d) Cows with ketosis are more

sensitive to insulin injections than normal cows (unpublished). (e) The blood glucose response to adrenalin injections is slight (1).

The following are not in accord with the view that an adrenal insufficiency is involved in the development of ketosis in dairy cows: (a) The plasma sodium and potassium appear to be normal (unpublished). (b) The intravenous glucose tolerance curve of cows with ketosis (1) is not typical of that of adrenal insufficiency in other species (3). (c) Cows with mild cases of ketosis and many with relatively severe cases frequently recover following a single intravenous injection of glucose.

Preliminary work indicates that both the plasma sodium and potassium are normal, although more data are needed to establish this with certainty. The secretion of the carbohydrate principle could still be affected abnormally, of course, without affecting the sodium and potassium relationship.

CONCLUSIONS

Four cows with ketosis, when treated with an extract of the adrenal cortex, responded immediately with an improvement in appetite and a return of the blood glucose and acetone bodies toward the normal values. However, an adrenal insufficiency is not necessarily believed to be indicated.

REFERENCES

(1) SHAW, J. C. Studies on Ketosis in Dairy Cattle. V. The Development of Ketosis. Jour. Dairy Sci., 26: 1079–1090. 1943.

(2) SHAW, J. C. Studies on Ketosis in Dairy Cattle. VIII. Spontaneous Recovery. Jour. Dairy Sci., 29: 151–155. 1946.

(3) THORN, G. W., KOEPF, G. F., LEWIS, R. A., AND OLSEN, E. F. Carbohydrate Metabolism in Addison's Disease. Jour. Clin. Invest., 19: 813–832. 1940.

THE INFLUENCE OF A SYNTHETIC THYROPROTEIN WHEN FED TO DAIRY COWS OVER AN EXTENDED PERIOD[1]

RALPH P. REECE

Department of Dairy Husbandry, New Jersey Agricultural Experiment Station, New Brunswick

INTRODUCTION

The development *in vitro* of a highly active thyroprotein by Reineke and Turner (2) has made possible the determination, by a number of investigators, of the influence of thyroprotein on milk secretion in dairy cattle. The results of these investigations rather consistently have shown an increase in milk and milk-fat production accompanied by an increase in heart rate and a decrease in body weight.

The feeding of a moderate daily dose of thyroprotein during a 3-week period was found to increase the fat content without greatly augmenting milk production (1). The present study was made to determine the influence of feeding thyroprotein over an extended period.

EXPERIMENTAL PROCEDURE

Nine dairy cows in various stages of declining lactation and gestation were fed daily either 10 or 15 g. of thyroprotein (Protamone[2]), which was incorporated in the grain ration. During the entire feeding period, daily milk weights (milking twice daily) were recorded, and milk samples were taken on two consecutive days each month. Individual milk samples were tested for their butterfat content by the Babcock method. Body weights and heart rates (measured with a stethoscope) were determined on two consecutive days each month. The part of the lactation during which thyroprotein was fed is compared with a similar segment of either a previous or a subsequent lactation. In giving information on reproductive performance, the last breeding date is listed as the date of conception. In tables 1 and 2 are included results from five cows used in the first experiment (1).

EXPERIMENTAL RESULTS

Immediate changes in milk production following the feeding of thyroprotein. Nine cows that received 10 g. of thyroprotein showed an average initial increase of 7.6 per cent, whereas five cows fed 15 g. of thyroprotein increased 19.7 per cent in milk production. These results are summarized in table 1.

313

TABLE 1 .

Immediate changes in milk production following the feeding of thyroprotein

Cow no.	Av. daily production (lbs.) for 7 days prior to feeding	Av. daily production (lbs.) for highest 7 days after feeding	% change in production
		10 g./day	
H37	29.3	34.0	+ 16.0
492	23.5	25.8	+ 9.8
383	22.3	25.6	+ 14.8
H41	15.2	15.7	+ 3.3
627	26.1	28.4	+ 8.8
621	64.3	66.8	+ 3.9
378	31.0	34.4	+ 11.0
485	23.0	24.2	+ 5.2
611	31.7	31.7	0.0
Av.	29.6	31.8	+ 7.6
		15 g./day	
628	15.8	21.5	+ 36.1
619	17.7	26.6	+ 50.3
H41	27.3	32.3	+ 18.3
492	29.1	32.5	+ 11.7
642	27.4	27.5	+ 0.4
Av.	23.5	28.1	+ 19.7

Milk production following the withdrawal of thyroprotein from the ration. Thyroprotein was withdrawn from the ration of five cows that had been receiving 10 g. per day. The average daily milk production for the last 7 days of feeding was 25.0 lbs. as compared with 20.1 lbs. for the second 7-day period after withdrawal, a decrease of 19.5 per cent. The daily milk production of three cows receiving 15 g. of thyroprotein decreased from 14.8 lbs. to 9.8 lbs., a decrease of 34.2 per cent following the withdrawal of thyroprotein from the ration (table 2).

TABLE 2

Milk production following the withdrawal of thyroprotein from the ration

Cow no.	Av. daily production (lbs.) for last 7 days of feeding	Av. daily production (lbs.) for second 7-day period after withdrawal	% change in milk production
	10 g./day		
H37	34.0	29.0	− 14.7
492	24.9	21.1	− 15.3
383	22.9	16.8	− 26.6
H41	14.6	10.3	− 29.5
627	28.4	23.3	− 18.0
Av.	25.0	20.1	− 19.5
	15 g./day		
619	10.9	4.6	− 57.8
628	10.9	9.8	− 10.1
621	22.7	14.9	− 34.4
Av.	14.8	9.8	− 34.2

Milk production following the withdrawal and the refeeding of thyro-protein. To determine whether thyroprotein was exerting a stimulating influence on milk secretion after a prolonged feeding period, it was withdrawn from the ration of four cows for 7 days and then included again. In each instance, a sharp decline in milk production followed the withdrawal of thyroprotein. When thyroprotein again was fed, three of four cows attained the pre-withdrawal level of milk production. The cow (378) that failed to return to the pre-withdrawal level of milk production had been receiving 40 g. of thyroprotein daily, and when refed was given only 15 g. daily. These results are summarized in table 3.

TABLE 3

Milk production following the withdrawal and the subsequent feeding of thyroprotein

Cow no.	Av. daily production by 7-day periods	Thyroprotein fed daily
	(*lbs.*)	(*g.*)
619	23.9	15
	19.5 (15.9*)	None
	17.9	15
	24.0	15
378	13.9	40
	13.1	40
	9.8 (5.5*)	None
	8.0	15
	7.4	15
611	13.1	15
	10.2 (7.1*)	None
	10.2	15
	13.2	15
642	22.1	15
	19.3 (16.6*)	None
	18.7	15
	22.1	15

* Lowest daily production for the period.

Influence of feeding thyroprotein from 3 to 17 months. A Holstein-Friesian cow (H–41; born 10–27–39; calved 2–7–43; conceived 4–27–43; aborted 8-month fetus 1–3–44; cow infected with *Brucella abortus*) received 15 g. of thyroprotein daily for 2.5 months. Milk production increased from 27.3 lbs. to 32.3 lbs. (table 1) and the butterfat percentage increased from 3.11 to 4.32 in the first month of the feeding period. Body weight increased during thyroprotein feeding, and heart rate showed a maximum increase of 16 beats per minute. Increasing the dose of thyroprotein from 15 to 30 g. for 15 days did not prevent the decline in milk production which was occurring at that time. Body weight had increased by 106 lbs. and heart rate had decreased by 14 beats per minute 22 days after the removal of thyroprotein from the ration. These results are summarized in table 4. In 3 months of a control lactation cow H–41, calving at 4 years and 2 months,

TABLE 4

Influence of thyroprotein in the ration of cow H–41

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1943)	(lbs.)	(lbs.)	(%)	(lbs.)	(per min.)	(g.)
Feb.	56.7	....	....	....	......	......
(19 days)						
March	68.9	2.43	3.53	....	......	......
April	66.1	1.98	3.00	....	......	......
May	64.6	1.74	2.70	....	......	......
June	45.5	1.25	2.75	....	......	......
July	34.1	1.06	3.11	1271	67	......
Aug.	29.3	1.27	4.32	1306	80	15
Sept.	22.8	0.95	4.20	1377	83	15
Oct.	12.7	0.58	4.59	1374	82	15*
Nov.	6.9	....	....	1480†	68†	......
(6 days)						

* Thirty grams of thyroprotein daily from Oct. 15 to Nov. 1.
† Determinations made 22 days following the removal of thyroprotein from the ration.

produced 1,853 lbs. of milk with a 3.58 per cent test; in a similar segment of a lactation following calving at 3 years and 3 months, she produced 1,984 lbs. of milk with a 4.33 per cent test when thyroprotein was fed.

An Ayrshire cow (485; born 5–18–38; calved 5–6–42; conceived 6–26–42; calved normally 3–28–43) was fed 10 g. of thyroprotein daily for 4 months. Milk production increased only slightly (table 1); however, the fat content of the milk increased markedly. Body weight decreased and then increased, and by the end of the feeding period the cow had gained 63 lbs. in body weight. Although heart rate varied, in 3 of the 4 months it was considerably higher than in the month prior to thyroprotein feeding. Table 5 presents a summary of these results. This cow calved at 3 years and 1 month

TABLE 5

Influence of thyroprotein in the ration of cow 485

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1942)	(lbs.)	(lbs.)	(%)	(lbs.)	(per min.)	(g.)
May	28.5	....	....	....	......	......
(20 days)						
June	26.0	....	....	....	......	......
July	22.9	0.93	4.04	....	......	......
Aug. (15 days)	23.3	0.92	3.94	1027	64	......
Aug. (16 days)	24.0	1.10	4.60	....	......	10
Sept.	21.3	0.98	4.58	980	78	10
Oct.	16.6	0.85	5.14	1013	65	10
Nov.	12.2	0.61	5.00	1033	92	10
Dec.	7.3	0.39	5.33	1090	86	10
(20 days)						

and in a 4-month segment of her lactation she produced 2,492 lbs. of milk
with a 4.14 per cent test. In a similar segment of a subsequent lactation,
calving at 3 years and 11 months, she produced 2,255 lbs. of milk with a 4.80
per cent test when fed thyroprotein.

A Brown Swiss, first-calf heifer (628; born 8–22–39; calved 9–28–42;
conceived 12–7–42; calved normally 9–16–43) received 15 g. of thyroprotein
daily for 4 months. This heifer had declined considerably in milk produc-
tion after calving and it was evident that she was increasing in body weight
at the expense of milk production. Milk production increased from 15.8 lbs.
to 21.5 lbs. per day (table 1), and in the first 3 months of thyroprotein feed-
ing this animal produced more milk than she had in the 3 months prior to
thyroprotein feeding. Although milk production did not decline sharply

TABLE 6

Influence of thyroprotein in the ration of cow 628

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyropro-tein fed daily
	Milk	Butterfat				
(1942)	*(lbs.)*	*(lbs.)*	*(%)*	*(lbs.)*	*(per min.)*	*(g.)*
Oct. (27 days)	24.7	1.19	4.80	....	....	....
Nov.	21.4	0.83	3.90	....	....	....
Dec.	17.9	0.83	4.65	....	....	....
(1943)						
Jan.	17.3	0.81	4.70	....	....	....
Feb.	16.2	0.75	4.64	....	55	....
March	19.5	0.97	4.98	1187	72	15
April	20.7	1.11	5.36	1126	66	15
May	20.5	1.02	4.98	1083	93	15
June	13.8	0.78	5.65	....	....	15
July	9.5	0.48	5.07	1137	78	....

after the withdrawal of thyroprotein (table 2), there were other indications
that the heifer had received stimulation from the thyroprotein in the last
month of the trial. The butterfat content of the milk increased following
the inclusion of thyroprotein in the ration and decreased when thyroprotein
was removed. From the first month to the third month of thyroprotein
feeding, there was a decrease of 104 lbs. in body weight. When the feeding
of thyroprotein was discontinued, body weight increased. However, 15 days
after the withdrawal of thyroprotein from the ration, body weight was still
50 lbs. below the weight prior to feeding. Heart rate increased by 17 beats
per minute during the first month of the feeding period and, following
thyroprotein withdrawal, a marked decrease in heart rate occurred. A sum-
mary of these results is given in table 6. This animal received thyroprotein
during 4 months of her first lactation, having calved at 3 years and 1 month.
In these 4 months she produced 2,274 lbs. of milk with a 5.21 per cent test.
In a similar segment of her following lactation, having calved at 4 years and
1 month, she produced 1,788 lbs. of milk with a 5.07 per cent test.

A Brown Swiss cow (619; born 4–19–36; calved 12–2–42; conceived 2–13–43; calved 11–30–43, the calf being dead when first observed) received 15 g. of thyroprotein daily for 4.5 months. Milk production increased from 17.7 lbs. to 26.6 lbs. per day (table 1). Thyroprotein was removed from the ration in the second month of the feeding period, and the average daily milk production decreased from 10.9 lbs. to 4.6 lbs. The fat content of the milk varied greatly but this was more apparent than real, since the cow was of a nervous nature and frequently failed to milk-out completely. A decrease in body weight was followed by an increase, the increase occurring when milk production was decreasing rapidly. The heart rate was increased by thyroprotein feeding and it remained above the pre-feeding level during the entire feeding period. These results are summarized in table 7. In 4 months of a

TABLE 7

Influence of thyroprotein in the ration of cow 619

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1942)	(lbs.)	(lbs.)	(%)	(lbs.)	(per min.)	(g.)
Dec. (27 days)	36.3	1.46	4.01	....	......	......
(1943)						
Jan.	36.7	1.14	3.10	....	......	......
Feb.	28.1	0.56	2.00	....	......	......
March	19.4	0.90	4.63	1222	59	......
(13 days)						
March	21.0	....	....	....	......	15 .
(18 days)						
April	25.2	0.99	3.92	1216	70	15
May	21.4	0.89	4.17	1141	71	15*
June	18.8	0.66	3.51	....	......	15
July	11.5	0.52	4.56	1178	75	15
Aug.	6.1	....	....	....	......	......
(18 days)						

* Thyroprotein withdrawn from ration for 7 days.

control lactation period, having calved at 5 years and 7 months, no. 619 produced 3,161 lbs. of milk testing 4.05 per cent. In a similar segment of a lactation period when thyroprotein was fed, this animal, calving at 6 years and 7 months, produced 2,341 lbs. of milk with a 4.00 per cent test.

An Ayrshire cow (492; born 1–11–39; calved 4–16–43; conceived 8–10–43; calved normally 5–19–44) received 15 g. of thyroprotein daily for 5.5 months. Milk production increased from 29.1 lbs. to 32.5 lbs. per day (table 1). The fat test increased from 3.28 per cent prior to thyroprotein feeding to 4.07 per cent in the third month of thyroprotein feeding. In the following 2 months the fat content of the milk decreased to the pre-feeding level, even though milk production was falling rapidly. The fat content of the milk showed an increase only when the stimulus to secrete milk was low. The decrease in the fat content of the milk was associated with an increase in

body weight. There was an initial loss in body weight; however, in the fifth month of thyroprotein feeding, no. 492 was 100 lbs. above her pre-feeding weight. Heart rate increased and then decreased. Table 8 presents a summary of these results. Similar segments of 5 months each of two lactations were available for comparison. Calving at 4 years and 3 months, no. 492 produced 3,274 lbs. of milk with a 3.82 per cent fat test when thyroprotein was fed; calving at 5 years and 4 months, she produced 2,912 lbs. of milk with a 3.30 per cent fat test when thyroprotein was not fed.

A Jersey cow (378; born 1–5–39; calved 9–1–42; conceived 2–25–43; aborted 7-month fetus 9–23–43; cow infected with *Brucella abortus*) was fed 10 g. of thyroprotein daily for 4 months, after which increasing amounts were fed until a dose of 40 g. was attained in the sixth month of thyroprotein

TABLE 8

Influence of thyroprotein in the ration of cow 492

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1943)	*(lbs.)*	*(lbs.)*	*(%)*	*(lbs.)*	*(per min.)*	*(g.)*
April (11 days)	33.5	....	....	....	....	......
May	39.6	1.36	3.45	....	....	......
June	35.7	1.12	3.15	....	....	......
July	30.9	1.01	3.28	1010	75	......
Aug.	31.0	1.18	3.80	989	82	15
Sept.	26.0	1.02	3.93	1006	76	15
Oct.	21.1	0.86	4.07	977	69	15
Nov.	19.6	0.74	3.75	1007	70	15
Dec.	9.4	0.30	3.22	1110	69	15
(1944) Jan. (13 days)	3.2	0.12	3.83	....	....	15

feeding. Milk production increased from 31.0 lbs. to 34.4 lbs. (table 1) soon after the inclusion of thyroprotein in the ration and then declined rather rapidly. However, this lack of persistency also was observed in a previous lactation. An attempt to prevent further decrease in milk production by increasing the thyroprotein dose did not meet with success. Butterfat percentage increased, but this increase probably was a result of the advance in the stage of lactation, since the fat content of the milk secreted during thyroprotein feeding was about the same as that secreted in a similar segment of a previous lactation. Body weight increased gradually until the last month of the feeding period. At that time body weight decreased slightly, and this decrease in body weight was accompanied by an increase in heart rate by 11 beats per minute. These results are summarized in table 9. Calving at 2 years and 8 months, no. 378 produced 2,757 lbs. of milk with a 3.97 per cent test in 6 months. In a similar segment of a subsequent lactation, calving at 3 years and 8 months, this animal produced 3,978 lbs. of milk with a 4.05 per cent test when thyroprotein was fed.

TABLE 9

Influence of thyroprotein in the ration of cow 378

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1942)	(lbs.)	(lbs.)	(%)	(lbs.)	(per min.)	(g.)
Sept. .... (20 days)	33.6	1.21	3.60	....	......	......
Oct.	31.9	1.12	3.52	830	82	......
Nov.	33.1	1.23	3.72	848	79	10
Dec.	26.8	1.10	4.12	859	82	10
(1943)						
Jan.	23.6	0.93	3.93	859	74	10
Feb.	18.8	0.82	4.38	878	74	10
March	15.5	0.66	4.26	889	76	*
April	13.6	0.57	4.22	872	87	*

* Thyroprotein gradually increased from 10 g. per day to 40 g. per day.

A Brown Swiss, first-calf heifer (642; born 11–8–40; calved 5–2–43; conceived 7–2–43; calved normally 5–11–44) was fed 15 g. of thyroprotein daily for 6 months. Milk production was not augmented (table 1); however, by the third month of thyroprotein feeding the fat test had increased from 3.51 to 4.12 per cent. In the second month of the trial, thyroprotein was withdrawn from the ration for 7 days. Milk production decreased from 22.1 lbs. to 18.7 lbs. (lowest daily production was 16.6 lbs.). The addition of thyroprotein to the ration resulted in the return of milk production to the prewithdrawal level (table 3). Increasing the grain allowance by 2 lbs. per day, in the fourth month of the feeding period, resulted in a further increase in the fat content of the milk. This increase in the fat content of the milk was accompanied by an increase in body weight. These results are presented in table 10. Similar segments of 6 months each, of two lactations, are avail-

TABLE 10

Influence of thyroprotein in the ration of cow 642

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1943)	(lbs.)	(lbs.)	(%)	(lbs.)	(per min.)	(g.)
May (25 days)	33.1	1.16	3.50	....	......	......
June	31.9	1.02	3.20	....	......	......
July	28.4	1.00	3.51	927	77	......
Aug.	25.6	0.98	3.84	910	63	15
Sept.	20.8	0.83	4.01	906	64	15*
Oct.	21.5	0.89	4.12	905	58	15
Nov.	21.7	0.99	4.58	972	59	15
Dec.	19.5	0.90	4.59	1027	61	15
(1944)						
Jan.	18.5	0.79	4.27	1088	62	15
Feb. (9 days)...	15.6	....	....	....	......	

* Thyroprotein withdrawn from ration for 7 days.

able for comparison.   Calving at 2 years and 6 months, no. 642 produced 3,909 lbs. of milk with a 4.22 per cent fat test when thyroprotein was fed; calving at 3 years and 6 months, she produced 6,039 lbs. of milk with a 4.03 per cent fat test when thyroprotein was not fed.

A Brown Swiss cow (621; born 12–10–36; calved 8–6–42; conceived 11–25–42; calved normally 8–31–43) received 10 g. of thyroprotein daily for 4 months and then 15 g. daily for 4 months.   There was an initial increase in milk production from 64.3 lbs. per day to 66.8 lbs. (table 1).   Fat production in the fifth month of thyroprotein feeding (eighth month of lactation) was similar to that in the month of peak fat production, which was just

TABLE 11

Influence of thyroprotein in the ration of cow 621

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1942)	(lbs.)	(lbs.)	(%)	(lbs.)	(per min.)	(g.)
Aug.	56.9	2.03	3.56	....	......	......
(23 days)						
Sept.	61.3	2.15	3.51	....	......	......
Oct.	66.4	2.27	3.42	1358	80	......
Nov.	64.7	2.25	3.48	1342	78	10
Dec.	61.5	2.48	4.04	1302	75	10
(1943)						
Jan.	59.3	2.40	4.04	1297	76	10
Feb.	55.9	2.09	3.73	1364	75	10
March	53.9	2.20	4.09	1324	76	15
April	50.6	1.95	3.86	1339	77	15
May	43.2	1.94	4.49	1339	75	15
June	29.7	1.41	4.74	....	......	15
July (14 days)	16.7	0.82	4.88	1397	68	......

prior to the addition of thyroprotein to the ration.   The fat content of the milk did not increase until the second month of thyroprotein feeding.   Body weight decreased slightly and then gradually increased.   By the seventh month of the feeding period, this animal had not attained a body weight equal to that recorded in the month prior to thyroprotein feeding; at that time she was in the sixth month of pregnancy.   There was no increase in heart rate.   It is believed, however, that heart rate was higher than it would have been had thyroprotein not been fed, since heart rate decreased following the removal of thyroprotein from the ration.   These results are summarized in table 11.   No. 621, calving at 5 years and 8 months, produced 12,671 lbs. of milk (4.00 per cent test) in 8 months when thyroprotein was fed.   In a similar segment of a subsequent lactation, calving at 6 years and 8 months, she produced 9,398 lbs. of milk (4.00 per cent test).

A Brown Swiss cow (611; born 8–24–34; calved 5–14–42; not pregnant) received 10 g. of thyroprotein daily for 6.5 months and 15 g. daily for an additional 10.5 months.   There was no increase in milk production, but there

was a substantial increase in the fat content of the milk.   Average daily fat
production remained fairly constant during the first 9.5 months of the feed-
ing period, after which it gradually decreased.   Increasing the dose of thyro-
protein from 10 to 15 g. per day did not increase either milk or milk-fat pro-
duction.   The withdrawal of thyroprotein from the ration for 7 days in the
fourteenth month of the feeding period resulted in a sharp decline in milk
production (table 3), indicating that thyroprotein had been stimulating milk
production.   The feeding of thyroprotein during the summer months did

TABLE 12

Influence of thyroprotein in the ration of cow 611

Date	Av. daily production		Fat test	Body weight	Heart rate	Thyroprotein fed daily
	Milk	Butterfat				
(1942)	(lbs.)	(lbs.)	(%)	(lbs.)	(per min.)	(g.)
May (9 days)	29.7	....	....	....	......	......
June	28.1	....	....	....	......	......
July	30.3	1.13	3.72	....	......	......
Aug.	32.5	1.25	3.85	1233	60	......
(15 days)						
Aug.	31.2	1.22	3.92	....	......	10
(16 days)						
Sept.	30.3	1.26	4.16	1171	63	10
Oct.	28.4	1.29	4.54	1169	59	10
Nov.	28.8	1.28	4.45	1180	72	10
Dec.	26.9	1.27	4.72	1192	60	10
(1943)						
Jan.	24.7	1.12	4.55	1185	58	10
Feb.	23.3	1.12	4.81	1208	53	10
March	23.5	1.15	4.90	1204	58	15
April	23.7	1.16	4.91	1195	66	15
May	23.9	1.14	4.78	1116	88	15
June	19.5	0.93	4.75	....	......	15
July	16.9	0.78	4.61	1159	87	15
Aug.	15.5	0.68	4.41	1182	59	15
Sept.	11.8	0.54	4.61	1207	59	15*
Oct.	12.6	0.60	4.77	1202	54	15
Nov.	12.1	0.59	4.89	1259	58	15
Dec.	8.2	0.39	4.80	1303	54	15
(1944)						
Jan. (11 days)	3.6	....	....	1387	64	15

* Thyroprotein withdrawn from ration for 7 days.

not prevent a decrease in fat test, although the fat tests probably were higher
than they would have been had thyroprotein not been fed.   Body weight and
heart rate fluctuated considerably, the lowest body weight being recorded
when the heart rate was highest.   Body weight remained below the pre-feed-
ing weight until the sixteenth month of the feeding period. · At the end of
the feeding period, at which time milk production was declining rapidly, the
cow weighed 154 lbs. more than she weighed prior to the feeding of thyro-
protein.   A summary of these results is presented in table 12.   It was pos-
sible to compare 305 days of the experimental lactation with a similar seg-

ment of a previous lactation.   Calving at 6 years and 6 months, no. 611 produced 6,704 lbs. of milk with a 4.39 per cent test in 305 days of a normal lactation; calving at 7 years and 8 months, she produced 8,161 lbs. of milk with a 4.55 per cent test in 305 days when thyroprotein was fed.

DISCUSSION

The feeding of 10 g. of thyroprotein daily to dairy cows resulted in an initial increase in milk production of 7.6 per cent.   Perhaps the most striking observation is the variation in response (from no response to +14.8 per cent).   One would anticipate, however, some variation in response, since the cows differed in age, stage of lactation, level of milk production, and breed. When 15 g. of thyroprotein were fed daily, the initial increase was more than twice as great as that obtained with 10 g.   In addition, the variation in response also was greater, from +0.4 to +50.3 per cent.

The per cent decrease in milk production on the withdrawal of thyroprotein from the ration was greater than the per cent increase following the feeding of thyroprotein.   The number of cows from which the averages were obtained, however, is not the same.   Five cows that received 10 g. of thyroprotein daily and from which the thyroprotein eventually was removed showed an average increase of 11 per cent in milk production following feeding and an average decrease of 19.5 per cent following removal.   In certain cases, after the withdrawal of thyroprotein from the ration, the level of milk production in the second week was lower than in the third week.   In one instance (no. 383), the average daily milk production for the second 7-day period after withdrawal was 16.8 lbs. but in the third week after removal it was 18.4 lbs.   The magnitude of the decrease in milk production following thyroprotein withdrawal appears to be associated with the stage of lactation and the degree of stimulation received during the feeding period. These observations suggest that, from the standpoint of milk and milk-fat production, after thyroprotein is included in the ration it should not be withdrawn until one desires to terminate lactation.   Lactation will cease rapidly following thyroprotein withdrawal.

Of the nine cows receiving thyroprotein from 3 to 17 months, six weighed more and three weighed less at the end of the feeding period than at the beginning.   In three instances in which observations were made, body weight increased rapidly following · thyroprotein removal.   Two of three cows that decreased in body weight during thyroprotein feeding failed to secrete milk with a higher fat content than that observed in a similar segment of a control lactation.   The question may be raised as to whether it is undesirable to have cows continue to lose in body weight after the peak of lactation is passed.   If this continued loss is undesirable, the additional feed intake required to prevent it must be determined.   Since body weight increases rapidly following thyroprotein withdrawal, it should not be too difficult to

attain the desired body weight before the next calving. Another point to consider would be whether the latter part of the lactation plus the dry period or the dry period alone is the most desirable time to replenish the body reserves of a cow.

Body weight losses may not be severe following thyroprotein feeding, but the fat content of the milk may decrease despite an initial increase (no. 492). Increasing the feed intake may result in a further increase in the fat content of the milk (no. 642). This suggests the desirability of increasing the feed intake of an animal when thyroprotein feeding is started if a maximum response is desired.

SUMMARY

The daily feeding of 10 g. of thyroprotein to nine cows resulted in an initial increase of 7.6 per cent in milk production; five cows receiving 15 g. of thyroprotein daily increased 19.7 per cent in milk production.

The average daily milk production in the second week after the withdrawal of thyroprotein was 19.5 per cent below that of the last week of thyroprotein feeding in five cows receiving 10 g. of thyroprotein; in three cows receiving 15 g. of thyroprotein the decrease was 34.2 per cent.

In three of four cows receiving thyroprotein, the withdrawal and refeeding of thyroprotein resulted in a marked decrease in milk production and then a return to the pre-withdrawal level. The cow not returning to the pre-withdrawal level was not fed thyroprotein at the previous level.

Of nine dairy cows that received either 10 or 15 g. of thyroprotein daily for periods varying from 3 to 17 months, six secreted milk with a higher fat content and six produced more milk than that secreted in a similar segment of either a previous or a subsequent lactation. Of the three cows not showing an increase in milk production during the entire thyroprotein feeding period, only one failed to show an initial response in milk production.

REFERENCES

(1) REECE, R. P. The Influence of a Synthetic Thyroprotein when Fed to Dairy Cows over a Three-week Period. Jour. Dairy Sci., 27(7): 545–550. 1944.

(2) REINEKE, E. P., AND TURNER, C. W. Formation in Vitro of Highly Active Thyroproteins. Their Biologic Assay, and Practical Use. Mo. Agr. Expt. Sta. Res. Bul. 355. 1942.

ASSOCIATION ANNOUNCEMENTS
PROGRAM
FORTY–SECOND ANNUAL MEETING
OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

ONTARIO AGRICULTURAL COLLEGE
GUELPH, ONTARIO, CANADA
JUNE 24–26, 1947

PROGRAM COMMITTEE

GENERAL:

J. A. NELSON, *Chairman*
Montana State College

G. E. RAITHBY
Ontario Agricultural College

MANUFACTURING:

B. E. HORRALL, *Chairman*
Purdue University

C. L. HANKINSON
North American Creameries, Inc.
Minnesota

F. J. BABEL
Iowa State College

W. C. LOY
Wilson and Co.
Illinois

EXTENSION:

W. T. CRANDALL, *Chairman*
Cornell University

E. H. LOVELAND
University of Vermont

A. R. PORTER
Iowa State College

PRODUCTION:

D. M. SEATH, *Chairman*
Louisiana State University

G. H. WISE
Iowa State College

G. W. SALISBURY
Cornell University

REGISTRATION

ADMINISTRATION BUILDING
ONTARIO AGRICULTURAL COLLEGE

Meetings will be held in the buildings on the campus of Ontario Agricultural College. Headquarters will be in the Administration Building.

PROJECTION EQUIPMENT

Lanterns will be available in all lecture rooms for projection of standard and $2'' \times 2''$ slides. Projectors for 16 mm. movies will be available by arrangement. Request for projection equipment should be made at the time abstracts of papers are submitted to the respective section Chairman. For the benefit of anyone bringing special electrical equipment, the available current is all 25 cycle.

COMMITTEE MEETINGS

Those wishing rooms for Extension and Production Section Committee meetings should write or contact G. E. Raithby and those in the Manufacturing Section wishing the use of rooms for Committee meetings should write or contact W. H. Sproule.

SPECIAL MEETINGS

Groups wishing rooms and equipment for special meetings before, during, or after the regular session will please contact G. E. Raithby. Provision also can be made for a limited number of breakfasts, luncheons, or dinners for special groups.

SCHEDULE OF PROGRAMS

Date and Time	General	Manufacturing	Production	Extension
Monday June 23, 1947	Registration			
Tuesday June 24, 1947				
8:00	Registration			
9:30–12:00	Opening Session			
1:00– 4:00		Papers	Papers	Papers
4:00– 5:00	Committees	Committees	Committees	Committees
8:00	Reception (Informal)			
Wednesday June 25, 1947				
9:00–12:00		Papers	Papers, A & B	Session
1:00– 1:30	Group Picture			
1:30– 5:30			Joint Meeting and Symposium	
1:30– 3:30		Papers		
3:30– 4:30		Business		
4:30– 5:30		Committees		
8:00	Mixer			
Thursday June 26, 1947				
9:00–11:00		Papers	Papers	Papers
11:00–12:00		Business	Business	Business
1:00– 3:00		Invitational Papers	Papers, A & B	
1:00– 2:00				Papers
2:00– 3:00				Business
3:00– 5:00	Business			
6:30	Annual Banquet			

GENERAL PROGRAM

Tuesday, June 24, 1947

Eastern Daylight Saving Time

9:30–12:00   OPENING SESSIONS, *War Memorial Hall*

     G. E. RAITHBY, *Department of Animal Husbandry,* presiding

     Introduction of Officers and Guests

     Address of Welcome

         W. R. REEK, *Acting President of Ontario Agricultural College*

     Presidential Address

         FORDYCE ELY, *President, American Dairy Science Association*

     Guest Speaker

         HON. GEORGE A. DREW, *K. C., Premier of the Province of Ontario*

     Announcements

1:00– 4:00   SECTIONAL MEETINGS

     Joint Meeting Production Sections A & B

         Field Husbandry Building

     Manufacturing Section

         Dairy Building

     Extension Section

         Animal Husbandry Building

4:00– 5:00   COMMITTEE MEETINGS

8:00   RECEPTION (INFORMAL)

Wednesday, June 25, 1947

9:00–12:00   SECTIONAL MEETINGS

     Production Section A

         Field Husbandry Building

     Production Section B

         Field Husbandry Building

     Manufacturing Section

         Dairy Building

     Extension Section

         War Memorial Hall

1:00– 1:30   GROUP PICTURE, *Administration Building*

1:30– 5:30   SECTIONAL MEETINGS

     Joint Meeting of Production Sections A & B and Extension Section

         War Memorial Hall

     Manufacturing Section

         Dairy Building

3:30– 4:30   BUSINESS MEETING
    Manufacturing Section
4:30– 5:30   COMMITTEE MEETINGS
    Manufacturing Section
8:00   MIXER

Thursday, June 26, 1947

9:00–11:00   SECTIONAL MEETINGS
    Joint Session Production Sections A & B
      War Memorial Hall
    Manufacturing Section
      Dairy Building
    Extension Section
      Animal Husbandry Building
11:00–12:00   BUSINESS MEETING OF SECTIONS
    Production Section
      War Memorial Hall
    Manufacturing Section
      Dairy Building
    Extension Section
      Animal Husbandry Building
1:00– 3:00   SECTIONAL MEETINGS
    Production Section A
      Field Husbandry Building
    Production Section B
      Field Husbandry Building
    Manufacturing Section
      Dairy Building
    Extension Section
      Animal Husbandry Building
3:00– 5:00   GENERAL BUSINESS SESSION
    War Memorial Hall
6:30   ANNUAL BANQUET
    Creelman Hall
    INSTALLATION OF OFFICERS AND PRESENTATION OF AMERI-
    CAN DAIRY SCIENCE ASSOCIATION AND BORDEN AWARDS

MANUFACTURING PROGRAM

Tuesday, June 24

Afternoon Session

Dairy Building

C. L. HANKINSON, *Chairman*

1:00– 4:00 **Bacteriology**

M1 Application of the Phosphatase Test to Various Dairy Products. GEORGE P. SANDERS AND OSCAR S. SAGER, *Bureau of Dairy Industry, U.S.D.A.*

M2 Time-Temperature Conditions Required to Inactivate Phosphatase in Different Dairy Products. GEORGE P. SANDERS AND OSCAR S. SAGER, *Bureau of Dairy Industry, U.S.D.A.*

M3 Determining the Germicidal Potency of Quaternary Ammonium Compounds and Their Use in Dairy Sanitation. W. S. MUELLER, D. B. SEELEY, AND E. P. LARKIN, *Massachusetts State College.*

M4 Effectiveness of Hypochlorite and Quaternary Ammonium Germicides in Destroying *Streptococcus agalactiae* in a Mastitis Sanitation Procedure. K. R. SPURGEON, P. R. ELLIKER, W. J. HARPER, AND J. R. FROEDGE, *Purdue University.*

M5 The Resazurin Reductase Test Using Prepared Sterile Dry Vials. N. S. GOLDING, *State College of Washington.*

M6 Some Observations on the Inversion of Sucrose by Invertase. L. E. MULL AND L. R. ARRINGTON, *University of Florida.*

M7 Observations on the Microscopic Analysis of Raw and Pasteurized Milk. G. H. WATROUS, JR., *Pennsylvania State College.*

M8 An Activity Test for Cheddar and Cottage Cheese Starters. B. E. HORRALL AND P. R. ELLIKER, *Purdue University.*

M9 Special Cultures for Manufacture of Blue Cheese from Pasteurized Milk. C. E. PARMELEE AND F. E. NELSON, *Iowa State College.*

M10 Lipase Production by *Mycotorula lipolytica.* I. I. PETERS AND F. E. NELSON, *Iowa State College.*

4:00– 5:00 **Committee Meetings**

Wednesday, June 25

Morning Session

Dairy Building

C. L. HANKINSON, *Chairman*

9:00–12:00 **Dry Milk Powders and Cheese**

M11 The Vapor Pressure–Moisture Relationships in Dry Milk Products. R. W. KUNKEL AND S. T. COULTER, *University of Minnesota.*

M12 Some Factors Influencing the Design of Spray Driers. ARNOLD KITZER AND S. T. COULTER, *University of Minnesota.*

M13 Changes Produced in Milk on Heating. H. A. HARLAND, R. JENNESS, AND S. T. COULTER, *University of Minnesota.*

M14 Some Changes in Dry Whole Milk during Storage. R. JENNESS, S. T. COULTER, H. A. HARLAND, AND L. K. CROWE, *University of Minnesota.*

M15 The Relationship of Ascorbic Acid to the Keeping Quality of Dry Whole Milk. S. T. COULTER AND R. JENNESS, *University of Minnesota.*

M16 Factors Affecting the Ease of Reconstitution of Milk Powders. U. S. ASHWORTH AND H. A. BENDIXEN, *State College of Washington.*

M17 The Use of Non-fat Dry Milk Solids for Mother and Batch Starters. B. E. HORRALL AND P. R. ELLIKER, *Purdue University.*

M18 Influence of Per Cent Oxygen in the Headspace Gas of Container on the Quality of Dry Whole Milk during Storage for One Year. G. H. WILSTER, *Oregon State College.*

M19 A Comparison of the Yields of Cheddar Cheese Obtained from Raw, Holder Pasteurized and High Short-Time Pasteurized Milk. O. W. IRVINE, L. R. BRYANT, D. C. HILL, AND W. H. SPROULE, *Ontario Agricultural College.*

Wednesday, June 25

Afternoon Session

Dairy Building

B. E. HORRALL, *Chairman*

1:30– 3:30 **Chemistry**

M20 A Study of the Volatile Acidity in Milk. P. G. MILLER, P. L. ZIMMERMAN, AND E. B. OBERG, *Carnation Company, Milwaukee, Wisconsin.*

M21 Some Unique Properties of Lactose as a Dietary Carbohydrate. LLOYD K. RIGGS, *Kraft Foods Company, Chicago, Illinois.*

M22 The Relationship between the Oxidation of Ascorbic Acid and the Development of the Oxidized Flavor in Milk. GEORGE R. GREENBANK AND PHILIP A. WRIGHT, *Bureau of Dairy Industry, U.S.D.A.*

M23 The Use of Carotene for Coloring Butter. G. A. RICHARDSON AND M. L. LONG, *University of California.*

M24 Graphic Procedure for Determining Quantitative Results of Volumetric Analysis. W. I. TRETSVEN, *Advisory Service, Chicago, Illinois.*

M25 Some Chemical Reactions Involved in the Production of the Sunlight Flavor in Milk. D. G. KEENEY AND D. V. JOSEPHSON, *Ohio State University.*

M26 A New Method for Determining Concentration of Quaternary Ammonium Germicide Solutions. W. J. HARPER AND P. R. ELLIKER, *Purdue University,* AND W. K. MOSELEY, *Indianapolis, Indiana.*

M27 Ionic Exchangers in the Dairy Industry. O. F. GARRETT, *M and R Dietetic Laboratories, Columbus, Ohio.*

3:30– 4:30 **Business Session**
4:30– 5:30 **Committee Meetings**

Thursday, June 26

Morning Session

Dairy Building

C. L. HANKINSON, *Chairman*

9:00–11:00 **Ice Cream, Evaporated Milk, and Sanitation**

M28 A Microscopic Study of the Texture of Ice Cream, W. S. ARBUCKLE, *University of North Carolina.*

M29 The Place of the Private Quality Control Laboratory in the Dairy Industry. M. A. COLLINS, *Dryden, New York.*

M30 Newly Developed Cleaning Aids for the Dairy Industry. JOHN R. PERRY, *Sealtest, Inc., New York, N. Y.*

M31 Some Observations on the Role of Sulphydryls in Heated Milk. STUART PATTON AND D. V. JOSEPHSON, *Ohio State University.*

M32 The Utilization of the Mineral-Ion Exchange Principle in the Manufacture of Evaporated Milk. D. V. JOSEPHSON AND C. B. REEVES, *Ohio State University.*

M33   The Manufacture of High-Solids Evaporated Milk.
      MARK KEENEY AND D. V. JOSEPHSON, *Ohio State University.*

M34   Condensing Whole Milk for Ice Cream Mix with the
      Vacreator.   G. H. WILSTER, *Oregon State College.*

M35   A Study of the Use of Nordihydroguairaitic Acid in
      the Storage of Frozen Sweet Cream.   J. W. STOLL,
      E. O. HERREID, AND P. H. TRACY, *University of
      Illinois.*

11:00–12:00   **Business Session**

Thursday, June 26
Afternoon Session
Dairy Building

C. L. HANKINSON, *Chairman*

1:00– 3:00   **Special Invitational Papers**
      Utilization of Whey.   B. H. WEBB, *Bureau of Dairy Industry, U.S.D.A.*

      Milk Lipase.   I. A. GOULD, *Dairy Department, University of Maryland.*

      Continuous Methods for the Manufacture of Butter.   A.
      W. FARRALL, *Agricultural Engineering, Michigan State
      College.*

      Physical Chemistry as Applied to Ice Cream Manufacture.
      H. H. SOMMER, *University of Wisconsin.*

3:00– 5:00   **General Business Session**
      War Memorial Hall

6:30   **Annual Banquet**
      Creelman Hall

PRODUCTION PROGRAM
Tuesday, June 24
Afternoon Session
Field Husbandry Building

DWIGHT M. SEATH, *Chairman*

1:00– 4:00   SECTIONS A & B, **Artificial Breeding, Heredity Studies,
Cross Breeding**
      P1   The Influence of Streptomycin upon the Livability
            and Bacterial Content of Bull Semen.   J. O. ALMQUIST, W. T. S. THORP, AND P. J. GLANTZ, *Pennsylvania State College.*

P2 Some Effects of Adding Thyroxine to Bull Semen. A. B. SCHULTZE AND H. P. DAVIS, *University of Nebraska.*

P3 Total Digestible Nutrients and Protein Levels for Dairy Bulls Used in Artificial Insemination. CECIL BRANTON, R. W. BRATTON, AND G. W. SALISBURY, *Cornell University.*

P4 A Study of Factors Affecting the Length of Gestation in Dairy Cattle. H. A. HERMAN AND R. W. SPALDING, *University of Missouri.*

P5 The Relationships of Frequency of Ejaculation, Age and the Ratio of Plasma Calcium and Phosphorus Levels to the Plasma Phosphatases of Dairy Bulls. J. T. REID, G. M. WARD, R. L. SALSBURY, AND C. E. SHUART, *New Jersey Agricultural Experiment Station.*

P6 Light Variation Associated with Conception Rate in Artificial Breeding. ERNEST MERCIER AND G. W. SALISBURY, *Animal Industry Service, Department of Agriculture of Quebec, Canada, and Cornell University.*

P7 Studies of Oxidative Mechanisms in Bull Semen. J. T. REID, R. L. SALSBURY, AND G. M. WARD, *New Jersey Agricultural Experiment Station.*

P8 Collecting Genetic Data through Cooperating Dairymen. N. P. RALSTON, S. W. MEAD, AND W. M. REGAN, *University of California.*

P9 Variation in the Type Ratings of Individual Ayrshire Cows. GEORGE HYATT, JR., AND W. J. TYLER, *West Virginia University.*

P10 Heritability of Type Ratings, and the Correlation between Type and Butterfat Production of Ayrshire Cows. W. J. TYLER AND GEORGE HYATT, JR., *West Virginia University.*

P11 Analysis of the Production Records of Cross-Bred Dairy Cattle. R. A. HILDER AND M. H. FOHRMAN, *Bureau of Dairy Industry, U.S.D.A.*

P12 Progress Report on Cross-Breeding of Dairy Cattle at Beltsville. M. H. FOHRMAN, *Bureau of Dairy Industry, U.S.D.A.*

P13 A Case of Intersex in Dairy Cattle. W. W. YAPP, *University of Illinois.*

4:00– 5:00 Committee Meetings

Wednesday, June 25

Morning Session

Field Husbandry Building

DWIGHT M. SEATH, *Chairman*

9:00–12:00   SECTION A, Feeding and Management

P14   Dairy Cattle Improvement Work of the Imperial Agricultural Research Institute—India. J. D. S. KUMARAN, *University of Missouri.*

P15   The Use of the Pen Barn as a Means of Mastitis Control. P. L. KELLY, D. F. BREAZEALE, G. S. HARSHFIELD, AND A. B. HOERLEIN, *South Dakota State College.*

P16   Report on Attempts to Prevent Mid-Summer Slump in Milk Production by Hay Feeding. D. M. SEATH AND G. D. MILLER, *Louisiana State University.*

P17   The Influence of Cracked Soybeans and Soybean Hay on the Flavor and Quality of Milk. ERLE E. BARTLEY AND C. Y. CANNON, *Iowa State College.*

P18   The Value of Adding Ground Alfalfa Hay to the Concentrate Mixture Fed with Prairie Hay in Rations for Dairy Cows. A. H. KUHLMAN AND H. W. CAVE, *Oklahoma A. & M. College.*

P19   The Effects of Calcium and Other Mineral Elements on the Lipid Partition in the Feces of Milking Cows. G. M. WARD AND J. T. REID, *New Jersey Agricultural Experiment Station.*

P20   Cobalt Tolerance in Young Dairy Cattle. H. A. KEENER, G. P. PERCIVAL, AND K. S. MORROW, *University of New Hampshire,* AND G. H. ELLIS, *U. S. Plant, Soil and Nutrition Laboratory, Ithaca, N. Y.*

P21   Phosphorus Metabolism Studies. I. Secretion of Phosphorus in Milk as Determined with the Radioactive Isotope. C. L. COMAR, W. A. KRIENKE, P. T. DIX ARNOLD, R. B. BECKER, AND GEORGE K. DAVIS, *University of Florida.*

P22   Feeding Value and Digestibility of Dehydrated Sweet Potatoes. L. L. RUSOFF, D. M. SEATH, AND G. D. MILLER, *Louisiana State University.*

GEORGE WISE, *Chairman*

9:00–12:00 SECTION B, **Colostrum, Mastitis, Milk Secretion**

P23 Studies on the Globulins of Bovine Colostrum. I. Isolation and Properties of a Water Soluble Globulin. R. G. HANSEN AND P. H. PHILLIPS, *University of Wisconsin.*

P24 Studies on the Globulins of Bovine Colostrum. II. The Absorption of Globulins by the Young Calf. R. G. HANSEN AND P. H. PHILLIPS, *University of Wisconsin.*

P25 Tocopherol Levels in the Colostrum and in the Early Milk of the Dairy Cow. D. B. PARRISH, G. H. WISE, AND J. S. HUGHES, *Kansas State College.*

P26 Action of Bacterial Filtrates Infused in the Mammary Gland. MAX L. DAWDY AND W. E. PETERSEN, *University of Minnesota.*

P27 The Effect of Intramammary Treatment for Mastitis upon Milk Production. ERIC W. SWANSON AND H. A. HERMAN, *University of Missouri.*

P28 The Use of Penicillin vs. Natural Recovery as a Means of Treatment of Mastitis. D. F. BREAZEALE, P. L. KELLY, G. S. HARSHFIELD, AND A. B. HOERLEIN, *South Dakota State College.*

P29 The Use of Non-Chlorine Disinfectants for the Washing of Cows' Udders. ROBERT H. KEITH AND PAUL M. REAVES, *Virginia Polytechnic Institute.*

P30 The Effect of Reduced Feed Intake on Mammary Growth and Lactation. J. F. SYKES, T. R. WRENN, AND S. R. HALL, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P31 Further Studies on the Effect of Vitamin D on Some of the Blood Changes in Normal and Milk Fever Cows at Parturition. J. W. HIBBS, W. D. POUNDEN, AND W. E. KRAUSS, *Ohio State University.*

P32 The Effect of the Quality and Quantity of Feed Pre-partum and Post-partum upon Blood Glucose and Blood Acetone Bodies. J. C. SHAW AND G. M. CAIRNS, *University of Maryland.*

P33 Vitamin A Deficiency in Dairy Cattle on Rations Containing Ground Raw Soybeans. J. C. SHAW, *University of Maryland,* AND L. A. MOORE AND J. F. SYKES, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

Wednesday, June 25

Afternoon Session

War Memorial Hall

W. T. CRANDALL AND D. M. SEATH, *Co-chairmen*

1:30– 5:30   **Joint Meeting with Extension Section**

Report of Dairy Cattle Health Committee, C. G. BRADT, *Chairman.*

Symposium—Brucellosis in Cattle.

3:00– 3:30   **Recess**

Report of Dairy Cattle Breeding Committee. E. J. PERRY, *Chairman.*

Report of Breeds Relations Committee. H. A. HERMAN, *Chairman.*

Symposium with PUREBRED DAIRY CATTLE ASSOCIATION participating, Getting the Most Out of Our Nation-Wide Testing Program.

Thursday, June 26

Morning Session

War Memorial Hall

DWIGHT M. SEATH, *Chairman*

9:00–11:00   SECTIONS A & B, **Roughage and Pasture**

P34   Values of Regular Corn Silage, Grainless Corn Silage and Ear Corn Silage in the Dairy Ration. KENNETH M. DUNN, RAY E. ELY, AND CARL F. HUFFMAN, *Michigan State College.*

P35   Carotene Losses from Artificially Dehydrated Alfalfa and from Artificially Dehydrated Alfalfa Silage. R. G. WASHBURN, W. E. KRAUSS, AND C. F. MONROE, *Ohio State University.*

P36   Silage Density: Effect of Pressure, Time and Crop Condition. A. E. PERKINS AND R. G. WASHBURN, *Ohio State University.*

P37   The Comparative Feeding Value of Corn Silage and Corn-Treated Meadow Crop Silage, with and without the Addition of Dilute Acetic Acid. C. F. MONROE, A. E. PERKINS, C. E. KNOOP, AND R. C. THOMAS, *Ohio State University.*

P38   The Digestibility of Coarsely Ground and Finely Ground Alfalfa for Dairy Heifers. ERIC W. SWANSON AND A. C. RAGSDALE, *University of Missouri.*

P39 Drying Requirements, Losses Due to Drying and Quality of Forage Obtained in Mow Hay Drying Experiments at Beltsville. J. B. SHEPHERD, L. G. SCHOENLEBER, H. G. WISEMAN, AND C. G. MELIN, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P40 Grazing Management Studies of Orchard Grass-Ladino Clover. P. S. WILLIAMS, V. G. SPRAGUE, C. B. KNODT, AND K. M. AUTREY, *Pennsylvania State College, and U. S. Regional Pasture Laboratory, State College, Pa.*

P41 The Evaluation of Several Grass-Legume Mixtures for Grass Silage and Aftermath Grazing. C. B. KNODT, V. G. SPRAGUE, P. S. WILLIAMS, AND K. M. AUTREY, *Pennsylvania State College, and the U. S. Regional Pasture Laboratory, State College, Pa.*

11:00–12:00 Business Meeting

Thursday, June 26

Afternoon Session

Field Husbandry Building

DWIGHT M. SEATH, *Chairman*

1:00– 3:00 SECTION A, Milk Secretion

P42 The Influence of Thyroprotein in the Ration of Dairy Cattle. RALPH P. REECE, *New Jersey Agricultural Experiment Station.*

P43 Some Effects of Feeding Thyroprotein to Dairy Cows. J. W. THOMAS AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*

P44 The Effect of Thyroprotein and Feed Intake on the Heart Rate of Dairy Steers. J. F. SYKES, T. R. WRENN, L. A. MOORE, AND J. W. THOMAS, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P45 Further Observations on the Effects of Feeding Thyroprotein to Dairy Cows. A. H. VAN LANDINGHAM, GEORGE HYATT, CHARLES E. WEAKLEY, JR., AND H. O. HENDERSON, *West Virginia University.*

P46 Absorption and Elimination of Thiouracil in Ruminants. RAY E. ELY, KENNETH J. OLSON, AND E. P. REINEKE, *Michigan State College.*

P47 The Influence of Thiouracil on Mammary Lobule-Alveolar Growth in Mice. JOHN P. MIXNER, *New Jersey Agricultural Experiment Station.*

P48 Factors Influencing the Male Hormone Content of Cow Manure. C. W. TURNER, *University of Missouri.*

P49 Progress Report in Study of Certain Goitrogens. G. W. PIPES AND C. W. TURNER, *University of Missouri.*

P50 The Effect of the Interval between Washing of the Udder and Attachment of Milking Machines upon the Bacterial Flora and Milk Production of Dairy Cows. C. B. KNODT, J. J. REID, P. S. WILLIAMS, AND E. M. KESLER, *Pennsylvania State College.*

GEORGE WISE, *Chairman*

1:00– 3:00 SECTION B, **Calf Feeding**

P51 The Riboflavin Content of Cow's Colostrum. T. S. SUTTON AND HAROLD E. KAESER, *Ohio State University.*

P52 Some Possible Relationships between Management, Fore-Stomach Contents, and Diarrhea in the Young Dairy Calf. W. D. POUNDEN AND J. W. HIBBS, *Ohio State University.*

P53 The Effect of Feeding Various Levels of Vitamin A on the Depletion Time of Dairy Calves. W. C. JACOBSON, H. T. CONVERSE, AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*

P54 The Antirachitic Properties for Calves of Hay Harvested by Field Curing, Barn Drying and Making Wilted Silage. L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*

P55 Changes in the Cell Volume and in the Concentration of Several Inorganic Constituents in the Blood of the Dairy Calf during Its Early Post-Natal Development. G. H. WISE, M. J. CALDWELL, D. B. PARRISH, AND J. S. HUGHES, *Kansas State College.*

P56 Distillers' Dried Solubles and Grains with Solubles as a Supplement in Dairy Calf Rations. J. R. SCHABINGER AND C. B. KNODT, *Pennsylvania State College.*

P57 The Utilization of β-Carotene, Vitamin A Alcohol, and the Natural Ester of Vitamin A by Holstein Heifers. R. H. ROSS, C. B. KNODT, AND N. B. GUERRANT, *Pennsylvania State College.*

P58   Soybean Oil Filled Milks for Feeding Young Dairy Calves.   NORMAN JACOBSON AND C. Y. CANNON, Iowa *State College.*

P59   The Placental Transfer and Colostral Storage of Vitamin D in the Bovine.   H. D. EATON, A. A. SPIELMAN, AND J. K. LOOSLI, *Cornell University.*

P60   The Effect of Protein Level on the Nitrogen Metabolism and Gains in Weight of Growing Holstein Calves.   G. P. LOFGREEN AND J. K. LOOSLI, *Cornell University.*

3:00– 5:00   **General Business Session**
War Memorial Hall

6:30   **Annual Banquet**
Creelman Hall

EXTENSION PROGRAM

Tuesday, June 24

Afternoon Session

Animal Husbandry Building

W. T. CRANDALL, *Chairman*

1:00– 1:15   **Opening Business Session**
1:15– 4:00   **Dairy Record Keeping**
Report of Dairy Records Committee.   J. F. KENDRICK, *Bureau of Dairy Industry, U.S.D.A.*
Canadian Registration and Production Policies.   G. E. Raithby, *Ontario Agricultural College.*
The Ohio Dairy Service Plan.   R. R. STARBUCK, *Ohio State University.*
Estimating the Yield of Pasturage on Farms (a) The Method; (b) Use in Dairy Herd Improvement.   R. E. HODGSON, F. J. ARNOLD, J. B. SHEPHERD, AND R. E. SHEAFFER, *Bureau of Dairy Industry, U.S.D.A. and the University of Maryland, cooperating.*

4:00– 5:00   **Committee Meetings**

Wednesday, June 25

Morning Session

War Memorial Hall

W. T. CRANDALL, *Chairman*

9:00–12:00   **Teaching Methods and Exhibits**
Technicolor, sound Extension Teaching Movie, ''The Challenge to New York Dairymen''.

Inspection of Exhibits and Explanation of Each Exhibit by Person in Charge.

Report of the Exhibit Committee.   L. A. JOHNSON, *Michigan State College.*

Wednesday, June 25

Afternoon Session

Animal Husbandry Building

W. T. CRANDALL AND D. M. SEATH, *Co-chairmen*

1:30– 5:30   **Joint Meeting with the Production Sections**
War Memorial Hall
Report of Dairy Cattle Health Committee.   C. G. BRADT, *Cornell University.*
Symposium—Brucellosis in Dairy Cattle.

3:00– 3:30   **Recess**
Report of Dairy Cattle Breeding Committee.   E. J. PERRY, *Rutgers University.*
Report of Breeds Relations Committee.   H. A. HERMAN, *University of Missouri.*
Symposium with PUREBRED DAIRY CATTLE ASSOCIATION participating, Getting the Most Out of Our Nation-Wide Testing Program.

Thursday, June 26

Morning Session

Animal Husbandry Building

W. T. CRANDALL, *Chairman*

9:00–11:00   **Artificial Insemination and Pen Stabling**
Selection and Repeatability of Sires Used in Artificial Insemination.   RAY ALBRECTSEN, *Cornell University.*
The Montana Elevated Cow Stall.   J. O. TRETSVEN, *Montana State College.*
The Uninsulated Pen Barn in Wisconsin.   G. R. BARRETT, *University of Wisconsin.*

11:00–12:00   **Business Meeting**

Thursday, June 26

Afternoon Session

Animal Husbandry Building

W. T. CRANDALL, *Chairman*

1:00– 2:00 4-H Club Programs

Report of 4-H Club Committee. G. W. VERGERONT, *University of Wisconsin.*

A 4-H Dairy Extension Program and Plan of Work. H. A. WILLMAN, *Cornell University.*

2:00– 3:00 Business Meeting

3:00– 5:00 General Business Session

War Memorial Hall

6:30 Annual Banquet

Creelman Hall

THE RELATIONSHIP OF THE PREPARTUM DIET TO THE CAROTENE AND VITAMIN A CONTENT OF BOVINE COLOSTRUM

A. A. SPIELMAN, J. W. THOMAS, J. K. LOOSLI, F. WHITING, C. L. NORTON, AND K. L. TURK

Department of Animal Husbandry, Cornell University, Ithaca

The purpose of the experiments reported in this paper was to determine the relationship of the prepartum diet to the carotene and vitamin A content of bovine colostrum.

The literature revealed scanty information concerning the effect of the feed of the dry cow upon the carotene and vitamin A content of colostrum. Kramer *et al.* (5) reported values of 25 and 28 I.U. of vitamin A per gram of colostrum from two cows on rye pasture and values of 16 and 20 I.U. for two cows receiving winter rations. Henry *et al.* (3) noted that cows on pasture before calving produced colostrum containing more carotene than barn fed cows, but that vitamin A was not affected. Stewart and McCallum (11) were unable to increase the vitamin A content of colostrum by feeding 3 lbs. of carrots or one-seventh of a pint of cod-liver oil per day to cows on winter rations. The paucity of available information and the practical importance of colostrum seemed to warrant further study of this problem.

PLAN OF EXPERIMENT

Twenty-nine Holstein and four Guernsey heifers in the Cornell University dairy herd were used in this experiment. These heifers had been on excellent pasture prior to the experiment and were considered to be in good physical condition. Approximately 60 days before calving they were divided into four dietary groups. The experimental diets were as follows: A low-carotene ration composed of wheat straw and a commercial grain mixture containing 12 per cent of protein; a standard dry-cow fitting ration consisting of the same grain mixture plus good quality hay and corn silage; a carotene-rich ration made by supplementing the standard dry-cow ration with one million I.U. of carotene[1] daily; and a vitamin A–rich ration made by

[1] Commercial concentrate containing 50,000 I.U. per gram, purchased from General Biochemicals, Inc., Chagrin Falls, Ohio.

adding one million I.U. of vitamin A[2] daily to the standard dry-cow ration. Periodic determinations of the potency of the carotene concentrate were made by measuring the absorption of a petroleum ether extract in an Evelyn photoelectric colorimeter at 440 mμ. Vitamin A potency of the shark-liver oil was determined by saponifying a weighed amount of the oil, dissolving the non-saponifiable portion in chloroform, and measuring the Carr-Price reaction in an Evelyn photoelectric colorimeter. The carotene content of the hay (U. S. No. 1 timothy-clover mixed) was 11.6 μg. per gram, and the corn silage contained 5 μg. of carotene per gram on the fresh basis as determined by a slight modification of the chromatographic method proposed by Charkey and Wilgus (2). Feed intakes were not recorded. The experimental rations were fed until the cows calved, after which a regular milking herd ration was fed.

Samples of colostrum were obtained on the first, third, and seventh days following parturition. The first sample, drawn shortly after parturition and before the calf had nursed, was obtained by pooling a pint of colostrum hand-milked from each of the four quarters of the udder. This sample was taken to represent the first colostrum as obtained by the calf following birth. After the first colostrum sample was taken, the cows were machine milked twice daily. All calves were removed from their dams at the end of the second day. The third and seventh day samples were taken from the sixth and fourteenth milkings postpartum. Carotene and vitamin A determinations were made according to the method of Boyer et al. (1).

RESULTS

The mean values with their standard error as well as the individual values in each dietary group are given in tables 1 and 2. The significance of mean differences was determined by a *t* test. *P* values of 0.05 arbitrarily are considered as significant, and *P* values of 0.01 are considered highly significant (6).

Carotene

The data presented in table 1 show the relationship of the prepartum diet to the carotene content of the colostrum. Carotene concentration is expressed per unit of volume and per gram of butterfat.

Pre-nursing sample. The difference between the means of the low-carotene group and the standard group is not significant, but the means of the carotene supplemented and the vitamin A supplemented groups are significantly higher when the results are expressed per gram of butterfat. The daily addition of one million I.U. of carotene to the standard fitting ration did not increase significantly the carotene content per 100 ml. of colostrum over that of the standard group. On the other hand, the mean value per

[2] Shark-liver oil, 41,000 I.U. per gram, generously supplied by National Oil Products Co., Paterson, N. J.

TABLE 1

The effect of the prepartum ration on the carotene content of colostrum

Cow no.	µg. per 100 ml.			µg. per gram butterfat		
	Pre-nursing	Third day	Seventh day	Pre-nursing	Third day	Seventh day
Low-carotene ration						
R-26	142	21	......	22.9	14.0	....
3	38	21	22	10.8	4.2	3.2
R-86	65	24	29	12.3	4.0	5.2
8	130	51	......	9.5	5.8	....
12	47	54	......	10.8	6.1	....
13*	187*	102*	29*	64.7*	20.6*	8.0*
17	45	19	15	14.5	4.4	2.9
R-95	169	12	5	18.2	4.5	1.0
24	42	28	5	11.3	2.6	0.9
Mean ± S.E.	85 ± 19	29 ± 5	15 ± 5	13.8 ± 1.6	5.7 ± 1.2	2.6 ± 0.8
Standard dry-cow ration						
1	208	50	21	35.4	9.6	3.9
7	32	61	19	21.4	9.2	3.7
14	170	57	19	22.9	8.1	2.5
R-74	121	40	13	33.5	9.2	3.4
23	70	27	25	15.0	6.5	3.1
34	38	58	38	23.0	8.9	9.2
Mean ± S.E.	107 ± 29	49 ± 4	23 ± 4	25.2 ± 3.1	8.6 ± 0.0	4.3 ± 1.0
Carotene-supplemented ration						
4	195	123	44	23.6	16.5	9.7
5	187	66	20	31.8	7.5	2.8
9	136	52	18	47.1	9.5	1.9
10	111	63	18	41.4	8.4	3.1
18	46	48	31	44.6	11.6	3.2
29*	198*	86*	41*	68.5*	16.0*	7.9*
30*	790*	127*	117*	173.9*	30.0*	19.5*
31	124	47	11	30.0	5.7	1.8
32	86	96	26	21.9	12.2	3.2
33	140	102	31	48.6	18.6	5.2
Mean ± S.E.	128 ± 17	75 ± 10	25 ± 4	36.1 ± 3.7	11.3 ± 1.3	3.9 ± 1.5
Vitamin A-supplemented ration						
2	56	105	26	40.2	12.7	6.2
R-64	100	61	15	28.5	11.4	3.1
6	72	38	26	24.1	6.3	3.1
16	400	25	1	61.5	3.5	1.7
20*	111*	80*	34*	71.7*	16.8*	5.4*
22	56	......	17	18.1	....	4.4
26	132	20	12	13.9	2.7	2.2
27	57	28	17	14.5	5.7	1.6
Mean ± S.E.	125 ± 32	46 ± 13	16 ± 0.04	28.7 ± 4.1	7.1 ± 1.6	3.2 ± 0.6

* Guernsey not included in mean.

gram of butterfat of the carotene-supplemented group is significantly higher (0.01 level) than that of the other groups.

Third-day sample. The increase in carotene content per unit of volume and per gram of butterfat of the carotene-supplemented group over that of

the other groups is highly significant. In contrast to the pre-nursing sample, the mean value of the low-carotene group is significantly lower than that of the standard group.

TABLE 2

The effect of the prepartum ration on the vitamin A content of colostrum

Cow no.	µg. per 100 ml.			µg. per gram butterfat		
	Pre-nursing	Third day	Seventh day	Pre-nursing	Third day	Seventh day
Low-carotene ration						
R–26	220	32	......	35.5	21.4	......
3	180	90	66	51.3	18.2	9.7
R–86	320	116	36	60.8	19.4	6.5
8	694	205	......	51.0	23.4	......
12	231	214	......	53.3	24.4	......
13*	86*	174*	11*	29.7*	35.1*	3.0*
17	172	64	47	55.6	14.7	9.2
R–95	188	62	45	19.6	23.1	9.6
24	216	150	24	58.1	14.0	4.3
Mean ± S.E.	278 ± 58	117 ± 23	44 ± 6	48.2 ± 4.5	19.8 ± 1.3	7.9 ± 1.1
Standard dry-cow ration						
1	418	88	29	71.1	16.9	5.4
7	182	97	12	121.6	14.7	2.3
14	735	202	70	98.9	28.8	9.4
R–74	160	71	33	44.3	16.4	8.6
23	626	117	98	134.8	28.3	11.9
34	124	163	84	75.9	25.0	20.4
Mean ± S.E.	374 ± 107	123 ± 21	54 ± 14	91.1 ± 13.8	21.7 ± 2.6	9.7 ± 2.5
Carotene-supplemented ration						
4	757	201	92	91.7	26.1	20.2
5	608	192	39	103.4	21.9	5.4
9	318	95	50	100.5	17.4	5.4
10	229	182	54	85.4	24.2	9.3
18	55	78	225	53.3	18.9	23.4
29*	335*	103*	44*	115.9*	19.2*	8.5*
30*	397*	57*	24*	87.4*	13.5*	4.0*
31	226	87	17	54.7	10.5	2.7
32	142	169	65	19.6	21.6	8.1
33	89	93	37	30.8	17.0	6.8
Mean ± S.E.	303 ± 87	137 ± 19	72 ± 23	67.4 ± 11.3	19.7 ± 1.9	10.2 ± 3.2
Vitamin A-supplemented ration						
2	199	896	98	142.8	108.5	23.4
R–64	239	216	62	68.1	40.3	12.8
6	429	323	43	143.4	54.0	5.1
16	5760†	249	67	885.9†	35.5	11.5
20*	309*	358*	101*	199.6*	75.4*	16.0*
22	962	....	79	310.7	....	20.7
26	1750	181	64	184.3	24.3	11.9
27	540	396	139	137.7	80.8	13.4
Mean ± S.E.	687 ± 210 1411‡	377 ± 108	79 ± 11	164.5 ± 32.9 267.5‡	57.2 ± 12.9	14.1 ± 2.3

* Guernsey, not included in mean.
† Unusually high value not included in mean.
‡ Including value †.

Seventh-day sample. Significant differences in the mean values of these samples are found only between the low-carotene group and the standard and carotene-supplemented groups. There is no difference per gram of butterfat.

Vitamin A

The effect of the prepartum ration on the vitamin A content of colostrum is shown in table 2.

Pre-nursing sample. There are no significant differences in the mean values of the low-carotene, the standard, and the carotene-supplemented groups when the results are expressed on a volumetric basis. On the other hand, per gram of butterfat, the difference between the low-carotene group and the standard and carotene-supplemented groups is highly significant. Colostrum from the cows receiving the vitamin A supplement contained 409, 313, and 384 µg. more vitamin A per 100 ml. and 116, 73, and 97 µg. more per gram of butterfat than that from the low-carotene, the standard, and the

TABLE 3

The relationship of the diet of the dry cow to the vitamin A potency of colostrum

Ration	Pre-nursing	Third day	Seventh day
	I.U./100 ml. *	*I.U./100 ml.* *	*I.U./100 ml.* *
Low-carotene ration	1245	516	201
Standard dry-cow ration	1674	574	254
Carotene-supplemented ration	1425	773	330
Vitamin A-supplemented ration	5850	1584	343

* Assuming 0.6 µg. carotene = 1 I.U.
   Assuming 0.25 µg. vitamin A = 1 I.U.

carotene-supplemented cows, respectively. These differences are highly significant. No explanation is apparent for the extremely high vitamin A value of the pre-nursing sample from cow no. 16.

Third-day sample. There is no apparent difference between the low-carotene, the standard, and the carotene-supplemented groups. The third-day sample of colostrum from the cows receiving the vitamin A supplement contained significantly more vitamin A than that from the other groups.

Seventh-day sample. Seven days after calving little difference existed between the vitamin A content of the colostrum from the cows receiving the low-carotene, standard, and carotene-supplemented rations. However, the effects of vitamin A supplementation still were evident, as indicated by the significant increase per gram of butterfat of the vitamin A–supplemented group over the other groups.

The total vitamin A potencies of the colostrum samples, expressed as I.U. per 100 ml., are given in table 3. These results are expressed on a volumetric basis as being representative of the vitamin A intake of the newborn calf. Colostrum from the vitamin A-supplemented cows contained 5,850 I.U. as

compared to 1,245 I.U. for the low-carotene group, 1,674 I.U. for the standard group, and 1,425 I.U. for the carotene-supplemented group. Effects of the vitamin A supplementation still were evident on the third day, this colostrum being approximately three times more potent in vitamin A than that from the low-carotene or standard group.

It was thought that the form of vitamin A present in the diet might be a factor in the mammary transmission of vitamin A. The data shown in table 4 indicate that the vitamin A present in colostrum is entirely of the ester form regardless of the prepartum ration. The analytical method used was essentially that described by Kascher and Baxter (4) for separating the ester and alcohol forms of vitamin A.

TABLE 4

The relationship of prepartum diet to the form of vitamin A in colostrum

Ration	Breed	Carote-noids	Vitamin A	
			Alcohol	Ester
		μg./100 ml.	μg./100 ml.	μg./100 ml.
Standard dry-cow	H	191	0	335
Carotene (alfalfa leaf meal)–supplemented	G	440	0	163
Vitamin A (ester form)–supplemented	G	724	0	1307
Vitamin A (ester form)–supplemented	H	136	2	213
Vitamin A (alcohol form)–supplemented	H	132	0	325
Vitamin A (alcohol form)–supplemented	H	158	0	251
Vitamin A (alcohol form)–supplemented	H	172	0	558

The plasma carotene and vitamin A values of the experimental cows 60 and 18 days before parturition have been reported (10). Using these values, highly significant positive correlations of 0.509 ± 0.14 and 0.523 ± 0.139 were found between the plasma carotene and vitamin A 18 days before calving and the carotene and vitamin A content of the pre-nursing samples of colostrum.

DISCUSSION

The value of colostrum in combating scours and allied diseases of new-born calves was demonstrated by Smith and Little (9). Later workers (8, 11) have associated the protective characteristics of colostrum with its high vitamin A content. The data presented here show that the pre-partum diet of the bovine may influence markedly the vitamin A activity of colostrum.

Large variations in the carotene and vitamin A levels were observed among these samples of colostrum. Undoubtedly sampling procedure was a factor. However, it is not known whether the butterfat content and the associated fat-soluble vitamins of colostrum vary with the completeness of milking, as is the case with normal milk. By expressing the carotene and vitamin A content on a per gram of butterfat basis, the variations due to

sampling should be minimized, since it seems unlikely that the first drawn sample should contain more or less of the fat soluble vitamins per gram of butterfat than the last drawn sample. Further study of this problem is needed.

The second finding in these studies is the lack of increase of vitamin A in the colostrum when one million I.U. of carotene was added daily to the standard dry-cow ration. These results are an interesting corollary to the relatively poor fetal storage of vitamin A in the newborn calves from these cows as previously reported (10). Specific differences in the efficiency of conversion of carotene to vitamin A, the stability of carotene in the digestive tract, and the action of vitamin E in conserving carotene and vitamin A may well be contributing factors.

Although it has been shown that the prepartum diet and the concentration of carotene and vitamin A in the blood stream prior to the decline at parturition influences the level in the colostrum, the processes involved largely are a matter of speculation. Liver reserves of carotene and vitamin A probably play a part and may explain the relatively high colostral carotene and vitamin A of the low-carotene group, since these heifers were on excellent pasture prior to the experimental period. The accumulation of milk in the udder prior to parturition and the observation of Petersen and Rigor (7) that milk left in the udder for several days assumes the composition of colostrum may indicate a simple storage phenomenon.

The question warrants further study as to whether or not supplementing the dry-cow ration with extra vitamin A will result in superior performance by the newborn calf or the cow.

SUMMARY

A study has been made of the relationship of the prepartum diet to the vitamin A and carotene content of bovine colostrum. Four different rations were fed to 29 Holstein and 4 Guernsey heifers during the last 60 days of their gestation periods. The rations were a low-carotene ration of wheat straw and a concentrate mixture; a standard dry-cow ration of concentrate, mixed hay and corn silage; the standard ration supplemented with one million I.U. of carotene daily; and the standard ration supplemented with one million I.U. of vitamin A daily.

Colostrum from cows receiving the low-carotene ration contained significantly less vitamin A per gram of butterfat than did colostrum from cows receiving the standard dry-cow ration.

The carotene content per gram of butterfat in the colostrum from the carotene-supplemented cows was significantly higher than that from the other groups, although the vitamin A content was not increased.

Colostrum from cows receiving the vitamin A supplement contained an average of 687 μg. per 100 ml. or 164.5 μg. per gram of butterfat, while the

colostrum from the standard dry-cow ration group contained only 374 μg. per 100 ml. or 91 μg. per gram of butterfat, showing that the vitamin A content of colostrum may be influenced by the prepartum diet.

Regardless of the form of vitamin A in the ration, the ester form of vitamin A predominated in the colostrum.

Highly significant positive correlations were found between the plasma carotene and vitamin A of the cows 18 days before calving and the carotene and vitamin A content of the colostrum.

REFERENCES

(1) BOYER, P. D., SPITZER, R., JENSEN, C., AND PHILLIPS, P. H.   Determination of Vitamin A and Carotene in Milk.   Indus. and Engin. Chem. (Analyt. Ed.), 16: 101–102.   1944.

(2) CHARKEY, L. W., AND WILGUS, H. S., JR.   Chromatographic Determination of Carotene in Alfalfa.   Indus. and Engin. Chem. (Analyt. Ed.), 16: 184–187.   1944.

(3) HENRY, K. M., HOUSTON, J., AND KON, S. K.   The Vitamin A and Carotene Content of Shorthorn Colostrum.   Jour. Dairy Res., 11: 1.   1940.

(4) KASCHER, H. M., AND BAXTER, J. G.   State of Vitamin A in Fish Liver Oils.   Indus. and Engin. Chem. (Analyt. Ed.), 17: 499–503.   1945.

(5) KRAMER, M. M., BAIR, M. D., KUNERTH, B. L., AND RIDDELL, W. H.   The Vitamin A Value of Colostrum and Milk of Four Cows Determined by the Single Feeding Method.   Jour. Agr. Res., 56: 227–232.   1938.

(6) LOVE, H. H.   Experimental Methods in Agricultural Research.   Agricultural Experiment Station, University of Puérto Rico, Rio Piedras, Puerto Rico.   1942.

(7) PETERSEN, W. E., AND RIGOR, T. V.   Relation of Pressure to Rate and Quality of Milk Secreted.   Soc. Expt. Biol. and Med. Proc., 30: 254–256.   1932.

(8) PHILLIPS, P. H., LUNDQUIST, N. S., AND BOYER, P. D.   The Effect of Vitamin A and Certain Members of the B-Complex upon Calf Scours.   Jour. Dairy Sci., 24: 977–982.   1941.

(9) SMITH, T., AND LITTLE, P. B.   The Significance of Colostrum to the Newborn Calf.   Jour. Expt. Med., 36: 181–198.   1922.

(10) SPIELMAN, A. A., THOMAS, J. W., LOOSLI, J. K., NORTON, C. L., AND TURK, K. L.   The Placental Transmission and Fetal Storage of Vitamin A and Carotene in the Bovine.   Jour. Dairy Sci., 29: 707–715.   1946.

(11) STEWART, J., AND MCCALLUM, J. W.   The Significance of the Vitamin A Content of the Colostrum as a Predisposing Factor in the Causation of Such Conditions.   Jour. Compar. Path. and Ther., 51: 290–295.   1938.

(12) STEWART, J., AND MCCALLUM, J. W.   The Effect of a Vitamin A Rich Diet on the Vitamin A Content of the Colostrum of Dairy Cows.   Jour. Dairy Res., 13: 1–4.   1942.

A STUDY OF A CREAM SEPARATOR BOWL WHICH IS CLEANED BY CENTRIFUGAL FLUSHING

E. O. HERREID, P. H. TRACY, R. V. HUSSONG, AND S. L. TUCKEY

Illinois Agricultural Experiment Station, University of Illinois, Urbana

In 1938 the W. and J. Whitehead, Ltd., in Laisterdyke, England, sent a centrifugal cream separator to the Illinois Agricultural Experiment Station. The bowl of this machine was designed to provide cleaning and drying by centrifugal force.[1] In 1945, another Whitehead separator was received by the Experiment Station. Both machines were investigated under various conditions of operation.

PRELIMINARY EXPERIMENTS

Tracy and Tuckey (8) in 1938 conducted a number of experiments with the English machine. The purpose of these early experiments was to study the automatic cleaning principle of this separator and ascertain its practical usefulness under farm conditions. The machine was used on two different farms near the Illinois Agricultural Experiment Station for periods of 5 to 7 days. Its operation also was studied in the dairy laboratories of the Experiment Station and results compared with those of a well-known American separator. The bacterial quality of the cream and skim milk was determined. The results of 24 separations, involving 12 comparisons, indicated that the English machine compared favorably with the American machine when both were operated under comparable conditions. This preliminary investigation indicated that the English separator was cleaned easily, that it was practical, that dairymen were interested in its development, and that the two farmers who used the separator were pleased with its performance. The separator was returned to England and World War II delayed further developments.

METHODS

In general, the washing procedure after each separation was as follows:
1. One pint of water at 100° F. was passed through the machine.
2. Next, 20 lbs. of water containing approximately 0.1 per cent of a

[1] The bowl has a specially constructed water distributor which allows cleaning solutions to flow from the supply tank to all parts of the bowl while it is in motion. In the shell of the bowl there are three discharge ports, equal distance apart, and each one is closed by a valve which is held in place by a steel spring. The tension in the steel spring is overcome by the centrifugal force so that the ports are tightly closed when the bowl revolves at its normal speed. When the speed slackens to one-half that of normal, the valves open and cleaning solutions can be forced through with turbulent cleaning action.

detergent[2] was flushed through the machine. This flushing was accomplished by first allowing the bowl to decrease in speed, which permitted the port valves to open; then about one-fourth of the water was allowed to run through. The speed of the bowl was increased, but not sufficiently to close the ports, and another one-fourth of the water was flushed through the bowl. This was repeated a third and a fourth time until all the water had been used.

3. The speed of the bowl again was increased to force out the last particles of water.

4. The supply tank and spout assembly were brushed after each separation (beginning with separation 37), using the detergent solution which previously had been flushed through the bowl; and finally rinsed with water at about 100° F.

5. Before separating each lot of milk, 25 lbs. of water at 165–170° F. was flushed through from the supply tank in about four equal portions. Samples of the whole milk, cream, and skim milk were taken with sterile pipettes, placed in sterile tubes, and cooled to about 35° F. with iced water. Plate counts for bacteria were made in duplicate, using tryptone-glucose-extract agar. The plates were incubated at 37° C., for 48 hours. Mold and yeast counts were made in duplicate on potato-dextrose-agar, acidified to pH 3.5 with tartaric acid. The plates were incubated 4 days at room temperature.

Fat tests were made by the Babcock method on the milk and cream and by the American Association method (3) on the skim milk in trials 2, 3, 4, and 5.

EXPERIMENTAL

In July, 1945, a new model of the English separator, slightly different in construction from the first one, was sent to the Experiment Station and subjected to more detailed investigation. In construction, the shell of the bowl and the water distributor were made of aluminum, while the cream screw and valve springs were made of brass and steel, respectively. The supply tank, spout assembly, and discs were not heavily tinned because of the existing tin shortage.

Testing the centrifugal flushing principle. The inventor had claimed that this machine could be cleaned by centrifugal flushing and that it was unnecessary to disassemble the separator for cleaning except at infrequent

[2] This detergent is called ''Vel'' and is the sodium salt of a sulfated monoglyceride. The coco-glyceride is chiefly used. The sodium sulfo-monoglyceride constitutes about 30–55 per cent on a dry basis; moisture 1–2 per cent; tetrasodium pyrophosphate 8–9 per cent, and the balance is mostly sodium sulfate, resulting from the reaction in obtaining the sulfated monoglyceride salt. This detergent was used in all trials and the authors are indebted to the Palmolive-Colgate-Peet Company for the supply used in the experiments reported in this paper.

intervals. The objective in this series of separations was to operate the machine each morning and evening over a period of 2 weeks without disassembling it and to determine the effectiveness of centrifugal flushing by measuring the quality of the cream and skim milk with the plate count method. The results are given in table 1. The machine was operated

TABLE 1

The effect on the bacterial plate counts of the cream and the skim milk of operating the English machine for 28 separations (Trial 1)

Separation no.	Time	Counts per ml. of:		
		Milk	Cream	Skim milk
25	a.m.	37,000	94,500	65,000
26	p.m.	37,100	96,000	13,700
27	a.m.	7,900	188,000	10,400
28	p.m.	8,600	146,000	7,800
29	a.m.	7,000	65,000	54,000
30	p.m.	4,200	1,630,000	*
31	a.m.	13,800	251,000	6,400
32	p.m.	7,600	1,700,000	14,500
33	a.m.	7,500	35,000	10,400
34	p.m.	8,000	1,250,000	8,500
35	a.m.	2,600	50,000	4,700
36	p.m.	2,600	1,300,000	257,000
37	a.m.	240,000	115,000	110,000
38	p.m.	215,000	174,000	130,000
39	a.m.	4,800	2,100	3,900
40	p.m.	6,200	2,000	32,000
41	a.m.	7,300	1,000	2,000
42	p.m.	9,900	26,000	4,900
43	a.m.	7,400	151,000	76,000
44	p.m.	7,600	10,000	9,700
45	a.m.	3,900	64,000	3,900
46	p.m.	5,500	9,800	47,000
47	a.m.	2,900	6,400	5,900
48	p.m.	3,600	4,900	7,500
49	a.m.	7,000	4,900	7,900
50	p.m.	7,000	10,000	9,700
51	a.m.	471,000	250,000	321,000
52	p.m.	449,000	414,000	806,000

* Sample lost.

without diassembling it through separation 36. The spout assembly was inspected and sour and putrid cream was found in the cream spout. Evidently the force of the washing solution from the bowl was not sufficient to remove adhering cream. Beginning with separation 37, the supply tank and spout assembly were washed by brushing each morning and evening with the water that previously had been flushed through the bowl. Including separation 38 and through the remainder of this trial, with the exception of separation 43, the plate counts of the cream do not reveal any significant contamination from one separation to the next. The only cream samples that revealed any yeasts and molds were those from separation 50, which showed 1 yeast and 1 mold colony; separation 51, which showed 1 yeast and

9 mold colonies; and separation 52, which showed 17 mold colonies per ml. The plate counts of the milk separated each morning and evening agreed closely, as expected, since the milk was obtained from the same lot.

The bowl was taken apart after separation 52. The lock ring had a bad odor as the result of milk solids which had accumulated in the threads, for it had not been tightened sufficiently at the beginning of the trial. The shell of the bowl was clean, but the dividing disc had some milk solids at the points opposite the holes in the discs. The top disc had a slight deposit of milk solids around each hole, and the amount of this deposit became progressively greater from the top to the bottom disc. The lower surface of the milk distributor had some adsorbed milk solids.

The inventor had claimed that the centrifugal flushing action from the

TABLE 2

The effect on the bacterial plate counts of the cream and skim milk of operating the English machine for 14 separations without disassembling the bowl, using a combination of detergents (Trial 2)

Separation no.	Time	Counts per ml. of:		
		Milk	Cream	Skim milk
53	a.m.	9,500	4,200	16,000
54	p.m.	13,000	3,500	18,000
55	a.m.	8,300	5,600	11,000
56	p.m.	9,800	7,600	20,000
57	a.m.	20,000	4,800	25,000
58	p.m.	260,000	8,000	40,000
59	a.m.	300,000	15,000	10,000
60	p.m.	15,000	4,000	*2,600,000
61	a.m.	8,000	1,400	5,500
62	p.m.	4,200	1,400	4,800
63	a.m.	11,000	*4,500	5,800
64	p.m.	10,500	*14,000	10,000
65	a.m.	120,000	*75,000	150,000
66	p.m.	15,000	50,000	332,000

* Spore-forming bacteria were present.

bowl also would be sufficient to clean the spout assembly. However, this series of separations proved conclusively that it is necessary to wash the supply tank and spout assembly after each separation. This was done in all the remaining trials.

In a second trial, the detergent "Vel" was combined equally by weight with another detergent and the mixture used in a concentration of about 0.1 per cent. The washing procedure was the same as that used in the previous experiment. The results are recorded in table 2. The machine was disassembled at the termination of the trial, and the underneath surfaces of all the discs had an adsorbed layer of white material which was, in all probability, a precipitate from the combined detergents. There was no separator slime on the inner wall of the bowl, but small amounts of solid materials were deposited near two of the discharge ports.

Washing the bowl without a detergent. A third trial was conducted to determine if the bowl could be washed without a detergent. Following the flushing of the bowl with the hot water, a pint of cold water was run through the machine to cool it so that milk would not adhere to the metal. The supply tank and spout assembly were washed as in previous trials, with the detergent solution. The milk separated each morning and evening was from the same lot.

The results in table 3 do not reveal any great increases in plate counts of the cream that could be attributed to contamination in the bowl from the preceding separation. The plate counts for bacteria in the cream and skim

TABLE 3

The plate counts of the cream and skim milk obtained with the English machine when no detergent was used to wash the bowl during 14 separations (Trial 3)

Separation no.	Time	Counts per ml. of:				
		Milk	Cream			Skim milk
		Bacteria	Bacteria	Yeasts	Molds	Bacteria
67	a.m.	49,000	42,000	......	5	96,000
68	p.m.	63,000	36,000	......	8	114,000
69	a.m.	142,000	106,000	1	16	170,000
70	p.m.	180,000	93,000	......	11	210,000
71	a.m.	10,000	4,200	2	6	14,000
72	p.m.	11,000	5,300	4	7	14,000
73	a.m.	4,500	4,100	2	4	6,600
74	p.m.	6,100	2,300	......	3	6,800
75	a.m.	71,000	4,200	1	5	86,000
76	p.m.	70,000	6,600	......	6	87,000
77	a.m.	2,800	9,200	......	9	51,000
78	p.m.	36,000	11,000	......	7	47,000
79	a.m.	230,000	129,000	1	2	389,000
80	p.m.	276,000	142,000	......	3	328,000

milk obtained from the duplicate lots of milk that were separated each day are in close agreement.

Examination of the various parts of the bowl revealed a very slight deposit of milk solids near the holes on the lower side of the first disc. This formation became progressively greater on each disc from top to bottom, whereas the upper surface of every disc was free from milkstone. The base of the bowl spindle was covered with a thin film of white material which was lightly adsorbed. The outer surface of the binding ring had a layer of milkstone, but there was no other deposit on the outside of the bowl.

Comparing the English and the American separators. A fourth trial was conducted to compare the quality of the cream and skim milk obtained by separating duplicate lots of milk with the English and the American separators. The American machine was washed after each separation by the rapid method suggested by Rudnick (7) except that 25 lbs. of water at

165–170° F. was passed through it immediately before each separation. The procedure for the English separator was the same as that outlined. Two 10-gallon cans of milk were obtained from the University dairy. The milk from one can was mixed well and divided equally each morning; one half of it was separated with the American and the other half with the English machine. The same procedure was repeated in the evening with the other can of milk, which had been held at 35–40° F. The results are summarized in table 4. The plate counts of the cream from separations 82 and 106 with the English machine are higher than the comparable plate counts from separations 81 and 105 with the American machine. The plate counts for

TABLE 4

Comparison of the bacterial plate counts of the cream and skim milk obtained by separating duplicate lots of milk with the American and English machines (Trial 4)

Separation nos.	Time	Counts per ml. of:				
		Milk	Cream		Skim milk	
			American	English	American	English
81,* 82†	a.m.	3,700	3,800	11,000	3,400	5,600
83, 84	p.m.	2,300	1,100	1,500	2,000	2,200
85, 86	a.m.	6,800	3,200	2,000	4,800	5,300
87, 88	p.m.	10,600	2,500	700	7,500	6,800
89, 90	a.m.	2,700	1,200	1,500	2,400	2,900
91, 92	p.m.	900	900	600	2,000	900
93, 94	a.m.	10,400	‡	‡	9,800	11,000
95, 96	p.m.	2,000	600	1,500	2,800	1,900
97, 98	a.m.	1,700	4,200	900	2,600	2,500
99, 100	p.m.	5,500	500	2,400	2,100	2,600
101, 102	a.m.	2,000	400	1,900	2,400	2,900
103, 104	p.m.	1,100	600	800	1,600	1,700
105, 106	a.m.	1,700	500	38,000	1,100	1,000
107, 108	p.m.	3,500	400	4,800	1,600	1,200

* Odd nos. represent separations with the American machine.
† Even nos. represent separations with the English machine.
‡ Samples lost.

the cream in the other 12 comparisons and those for all the skim milks obtained with both machines agreed closely.

The bowl of the English machine was sanitary at the end of the trial except for the three bottom discs, which showed slight traces of dried milk solids. There was not the slightest trace of milk solids on either the inner shell or the outer surface of the bowl. The rapid procedure used to wash the American machine did not remove all milk solids from the lower surfaces of all the discs.

Separating milk of inferior quality. The milk separated with the English machine in the preceding trials was of good quality, as indicated by relatively low plate counts. It was deemed advisable to determine the effect of separating milk of poor quality on the plate counts of the cream

and skim milk and on the centrifugal flushing ability of the bowl in the English machine. Two 10-gallon cans of milk were obtained and dumped into a vat, mixed well, and poured back into the same cans. One can of milk was separated immediately and the other was held at 36–40° F. and separated in the evening. The washing procedure was the same as that outlined.

With the exception of separation 112 (table 5) there was little evidence of contamination from one separation to the next, as indicated by the bacterial plate counts of the cream. Except for separations 109 and 110, there is fairly close agreement in the plate counts of the skim milk each morning and evening from the duplicate lots of milk. The milk used in separations

TABLE 5

The effect on the plate counts of the cream and skim milk of separating milk of poor quality with the English machine (Trial 5)

Separation no.	Time	Counts per ml. of:				
		Milk	Cream			Skim milk
		Bacteria	Bacteria	Yeasts	Molds	Bacteria
109	a.m.	12,500,000	1,650,000	2	6	2,220,000
110	p.m.	16,000,000	3,100,000	....	....	23,000,000
111	a.m.	20,000,000	6,400,000	2	12	21,500,000
112	p.m.	32,500,000	18,400,000	100	2	14,700,000
113	a.m.	1,200,000	1,500,000	130	100	4,700,000
114	p.m.	5,800,000	1,450,000	80	10	4,100,000
115	a.m.	4,200,000	9,300,000	4	7	5,500,000
116	p.m.	4,400,000	7,200,000	4	5	3,900,000
117	a.m.	300,000	250,000	9	2	330,000
118	p.m.	430,000	220,000	4	5	450,000
119	a.m.	1,000,000	460,000	12	7	1,300,000
120	p.m.	1,500,000	420,000	250	3	1,000,000
121	a.m.	5,800,000	8,000,000	12	2	8,200,000
122	p.m.	3,200,000	6,500,000	5	3	8,600,000

119 and 120 showed evidence of mastitis, as indicated by the presence of dark-colored coagulated material on a sediment disc. The inner shell of the bowl was inspected and found to be clean before proceeding with separation 119. Immediately after completing separation 120 and after washing by the prescribed procedure, dark material was found adsorbed on the inner shell of the bowl. This material had accumulated during separations 119 and 120 and was not removed by the washing procedure. A sediment test was made of the milk used in separations 119 to 122, inclusive, and the disc scored 6.5 and 7, which is indicative of the poor quality of the milk. At the conclusion of the trial the three bottom discs had small amounts of adsorbed milk solids, but the others were bright and clean on the upper and lower surfaces.

In trial 5, the fat content of the milk averaged 4.6 per cent and that of the resulting cream, 57.43 per cent. The cream contained a higher per-

centage of fat in this trial because the screw on the bowl did not permit a
sufficient range of adjustment for milk of high fat content to obtain cream
of what might be called normal fat content. In spite of the high fat con-
tent of the cream, the bowl was remarkably well cleaned up to and including
separation 118, as indicated by the appearance of the inner shell.

Distribution of bacteria in the cream and the skim milk. This study
provided data on the distribution of the bacteria in the cream and skim
milk. The results from trials 2, 3, 4, and 5 are shown in table 6; trial 1 was
excluded because it was preliminary. In 85 per cent of the separations
with the English machine, the cream had a lower plate count for bacteria
than the skim milk; in 80 per cent of the separations the cream had a lower
count than the original milk. Similar results were obtained by Tracy and
Tuckey (8). The results in table 4 also show that the cream obtained with

TABLE 6

*Comparative distribution of the plate counts of the milk, cream, and skim milk
for 55 separations with the English separator*
(Trials 2, 3, 4, and 5)

Plate counts of:	Total	
	No.	%
Cream lower than skim milk	47	85
Cream higher than skim milk	8	15
Cream lower than milk	44	80
Cream higher than milk	11	20

the American machine had a lower plate count than the skim milk in 11
of 13 comparisons. These data agree with the results of seven separations
reported by Ulvin and Cree (9). The other data reported in the literature
do not agree on the distribution of bacteria in cream and skim milk obtained
by centrifugal separation. Eckles and Barnes (2) and Anderson (1) re-
ported higher plate counts in cream than in skim milk. The results of
Lamson (5) indicate less or only slightly more bacteria in separated cream
than in the whole milk, but that the skim milk contained fewer bacteria than
the whole milk. Leete (6) in 100 separations did not find much difference
in the bacterial counts of cream, skim milk, and whole milk, the average
counts being 501,000, 313,000, and 435,000 per ml., respectively.

The factors which determine the distribution of bacteria in cream and
skim milk obtained by centrifugal separation should be studied in more
detail because of the implications in determining bacterial standards for
market cream. It is probable that the physical state of the fat globules
and their membrane materials, the temperature of the milk, and the types,
numbers, and specific gravity of the microorganisms are factors which deter-
mine their retention by either the cream or the skim milk.

Skimming efficiency. The skimming efficiency of the English machine
was determined in trials 1, 2, 3, 4, and 5. The results assembled in table 7

TABLE 7

The milk fat content of the skim milk from 84 separations with the English machine

% milk fat in skim milk	No. of separations
0.03	1
0.04	2
0.05	8
0.06	11
0.07	20
0.08	14
0.09	11
0.10	6
0.11	2
0.12	3
0.13	1
0.14	0
0.15	3
0.16	0
0.17	0
0.18	0
0.19	0
0.20	2
Mean 0.08	84

indicate an average of 0.08 per cent fat in the skim milk, which compares favorably with results reported in the literature (4) of 0.06 to 0.08 per cent fat in the skim milk for a factory separator. In trial 5, 14 comparisons were made with the American and English machines. While the fat tests of the skim milk were slightly lower for the American machine, the data are insufficient to draw definite conclusions.

Time required to wash the English separator. Washing this separator by centrifugal flushing of the bowl, including brushing and rinsing the supply tank and spout assembly, varied from 3.5 to 5.5 minutes, depending on the experience of the operator. A person accustomed to the procedure can wash this machine after each separation in about 3 minutes and assemble it for operation in about 0.5 minute. When each experimental trial was terminated, the supply tank, spout assembly, and the bowl were disassembled, the discs inspected and washed, and the machine reassembled. The time required for this operation for one person for whom records are available was 17 minutes.

DISCUSSION

The experiments reported indicate that the English separator cannot be cleaned entirely by centrifugal flushing, as originally claimed by the inventor. However, the bowl can be washed easily and quickly without disassembling it; the supply tank and spout assembly must be washed after each separation. The bowl was cleaned remarkably well by the flushing action of the washing solutions, even though it was necessary to use a mild detergent of low alkalinity because of the aluminum bowl and some of its

parts and because of the inadequately tinned discs, supply tank, and spout assembly.

The fact that the plate counts of the cream were lower than those of the skim milk in 85 per cent of the separations is of interest, but it is questionable if these results can be attributed to any superiority of the English machine as similar results were obtained in a few trials with the American machine and in a limited number of trials by other investigators (9).

The skimming efficiency of the English machine of 0.08 per cent fat in the skim milk, using the American Association method (3), compares favorably with results reported for a factory separator.

This separator has been demonstrated to be practicable for farm use. Furthermore, the useful life of the bowl will be prolonged because it will have to be disassembled and washed less frequently than present machines.

CONCLUSIONS

1. The bowl of the English separator can be washed properly by centrifugal flushing with two separations daily over a period of 1 to 2 weeks.

2. In the great majority of cases, there was no significant contamination from one separation to the next, as indicated by the plate counts of the cream and of the skim milk.

3. Presence of 0.08 per cent fat in the skim milk from the English separator as determined by the American Association method compares favorably with results obtained with a factory separator.

4. The plate counts of the cream were lower than the skim milk in 85 per cent of the cases and lower than the milk in 80 per cent of the cases.

REFERENCES

(1) ANDERSON, J. F.   The Relative Proportion of Bacteria in Top Milk and Skim Milk and Its Bearing on Infant Feeding.   U. S. Pub. Health and Mar. Hosp. Serv. Hyg. Lab., Bul. 56.   1909.

(2) ECKLES, C. H., AND BARNES, S. E.   Purification of Milk by Centrifugal Separation. Iowa Agr. Expt. Sta. Bul. 59.   1909.

(3) HANSEN, H. C., HORRALL, B. E., BIRD, E. W., AND JENSEN, C.   Final Report of Subcommittee on the Determination of the Percentage of Fat in Buttermilk, Skim Milk and Whey.   Jour. Dairy Sci., 28: 325–327.   1945.

(4) HERREID, E. O., WHITMAN, D. W., AND SLACK, R. O.   Accounting for Milk Fat.   Vt. Agr. Expt. Sta. Bul. 453.   1944.

(5) LAMSON, R. W.   A Comparison of Bacteria Counts in Whole and Skim Milk, Separator and Centrifugal Cream.   Jour. Dairy Sci., 1: 498–507.   1918.

(6) LEETE, C. S.   Relation between the Bacterial Count of Whole Milk and That of the Cream and Skim Milk Separated from It.   Jour. Agr. Res., 31: 695–699.   1925.

(7) RUDNICK, A. W.   New Method of Washing Cream Separators in Two Minutes. DeLaval Monthly, 37(1): 7.   1943.

(8) TRACY, P. H., AND TUCKEY, S. L.   Unpublished Results, Ill. Agr. Expt. Sta.   1938.

(9) ULVIN, G. B., AND CREE, N. F.   Standard Plate Counts on Raw Milk and on the Raw Cream and Raw Skimmed Milk Obtained from It.   Jour. Milk Tech., 8: 257–263. 1945.

THE EFFECT OF SULFANILAMIDE IN THE DILUENT UPON FERTILITY OF BULL SEMEN

G. W. SALISBURY AND C. B. KNODT[1]

Laboratory of Animal Breeding and Artificial Insemination,
Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

Since 1939, when the possible effects of bacteria on the results of metabolic studies with spermatozoa or on the results of artificial insemination were first discussed by Salisbury *et al.* (9), this laboratory has been engaged in seeking a solution to that problem (3, 4, 5, 8). Recently, Knodt and Salisbury (5) have shown that 300 mg. of sulfanilamide added per 100 ml. of yolk-citrate diluent resulted in significant improvements in the livability of bull spermatozoa stored in this diluent. The sulfanilamide effectively controlled most of the bacteria normally found in semen and also depressed carbohydrate and oxygen utilization, but it increased the accumulation of lactic acid. Whether or not the stimulation of the spermatozoa to greater livability was due to the control of bacteria or to the effect on their metabolism was not proved.

The present paper deals with the fertility of bull spermatozoa when diluted with yolk-citrate to which sulfanilamide was added at the rate of 300 mg. per 100 ml.

EXPERIMENTAL

Three different experiments were conducted to determine the effect of sulfanilamide on fertility. Each was planned so that the diluent treatments were effectively randomized among the samples of semen collected and used from each experimental bull. The bulls used were those owned by the New York Artificial Breeders' Cooperative, Inc., and inseminations were made by the regularly employed inseminators on cows owned by the members.

The first experiment. The first study was conducted in June, 1945. It was designed to compare the regular yolk-citrate diluent with a yolk-citrate-sulfanilamide diluent containing 300 mg. of added sulfanilamide per 100 ml. and, also, a yolk-citrate-glucose diluent containing 540 mg. of added glucose per 100 ml. The latter diluent was used to determine the effect of added glucose upon fertility, for it had been shown earlier (10) that glucose effectively increased livability of and lactic acid production by bull spermatozoa.

The citrate buffer containing 3.6 g. of sodium citrate (dihydrate) per 100 ml. was prepared with water distilled in glass and autoclaved for 20 minutes at 15 lbs. pressure. Glucose was made up in approximately

isotonic solutions (5.4 per cent) and sterilized in the same way as the citrate solutions. The yolk-citrate-glucose diluent was prepared by adding 20 ml. of isotonic glucose solution to 80 ml. of citrate buffer, and this mixture was added to 100 ml. of yolk to give a level of 540 mg. of glucose per 100 ml. Various multiples of these proportions were utilized, depending upon the total need for diluent.

The citrate-sulfanilamide solution was prepared by heating water distilled in glass to approximately 80° C. and then dissolving in it the desired amount of sulfanilamide and citrate to give a final concentration of 3.6 g. sodium citrate (dihydrate) and 600 mg. of sulfanilamide per 100 ml. This solution was made up to a predetermined volume before autoclaving at 15 lbs. pressure for 20 minutes. Equal volumes of yolk and citrate-sulfanilamide solution then were mixed for the final diluent. In the first investigation, all diluents were made up before the investigation started and no precautions were taken to prevent direct light from falling on the citrate-sulfanilamide diluent during the 26 days which were required to complete the collections.

Ten bulls were used, 5 Holsteins and 5 Guernseys. Three collections at intervals of 1 week to 10 days were taken from each bull, and the diluent to be used for any particular collection for any one bull was assigned at random. Thus each bull was a block and the design was that of a randomized block.

All semen was diluted so that each milliliter of diluted semen contained 30 million spermatozoa. This was done in order not to have dilution rates as a confounding factor. Later (7) it was found that this precaution was unnecessary within the limits of dilution used and it was not continued in the two subsequent experiments. Semen was shipped to 47 member units of the Cooperative.

Table 1 shows the data for the average quality characteristics of the semen used with each diluent. Statistical analysis of the data showed no significant differences between the semen samples used for each treatment. As can be seen, additions of glucose apparently increased methylene blue reduction time, but this difference was not statistically significant. Also, the results of the inseminations are shown in table 1. It is obvious that no difference in fertility was found between the yolk-citrate and the yolk-citrate-sulfanilamide diluents in this experiment.

In view of our more recent investigations, the authors are of the opinion that as a consequence of storing in clear bottles on the laboratory desk, the sulfanilamide in the alkaline citrate (pH about 7.45) was oxidized by the action of light, as evidenced by a slight browning, and thereby lost its effectiveness. This view is supported by data from a later experiment in which a limited number of ejaculates were treated with freshly prepared citrate-sulfanilamide as compared to the citrate-sulfanilamide diluent which

TABLE 1

Average semen quality characteristics and fertility results in the first experiment

Semen character	Diluent used		
	Yolk-citrate	Yolk-citrate-glucose	Yolk-citrate-sulfanilamide
Concentration 1,000's/mm.^{3}	1,232	1,280	1,190
Motility, %	80.0	79.0	79.0
Methylene blue reduction time, min.	3.88	4.68	4.08
Fertility			
Services, no.	786	720	767
5-mo. N.R.,* no.	377	352	368
5-mo. N.R., %	48.0	48.9	48.0

* N.R. = non-returns to service.

had been exposed to light. The action of light brought about changes in the sulfanilamide solution so that it failed to stimulate spermatozoan livability.

The addition of glucose to the citrate resulted in a slightly smaller proportion of returns to service during the 5 months following service, but the difference was not mathematically significant. There was some evidence in the data that the samples to which the glucose was added had been used more days in the field than the other samples, but again this difference was not statistically significant.

The second experiment. The second fertility experiment was conducted in November, 1945. The design was essentially the same as in the first experiment. Collections from ten bulls, 5 Holsteins and 5 Guernseys, were made twice with an interval of 7 to 11 days. To one or the other of these collections the diluent to be used, yolk-citrate or yolk-citrate-sulfanilamide,

TABLE 2

Average semen quality characteristics and fertility results in the second experiment

Semen character	Diluent used	
	Yolk-citrate	Yolk-citrate-sulfanilamide
Concentration 1,000's/mm.^{3}	1,189	1,187
Motility, %	79.5	78.5
Methylene blue reduction time, min.	4.82	4.88
Fertility		
Services, no.	676	765
5-mo. N.R.,* no.	379	476
5-mo. N.R., %.	56.1	62.2

* N.R. = non-returns to service.

was assigned at random. In this investigation the two buffers for the diluents were made up and sterilized as described above, but were stored in the dark during the 18 days required to complete the semen collections. The diluted semen was shipped to 66 local units of the Cooperative. Table 2 gives the average semen quality characteristics and the results of the inseminations.

' In this experiment the results were strikingly different from those obtained in the first one. An analysis of covariance, using the number of services as the independent variable and 5-month non-returns as the dependent variable, indicated that the 6.1 per cent difference in level of fertility for the diluents was mathematically significant at the 5 per cent level of probability.

The varying results of the first and second experiments suggested that the different fertility levels observed might be due simply to the random variability typical of biological experiments. The number of sulfanilamide-treated semen samples was small, only 10, and the level of odds for the differences in fertility was just barely over the 5 per cent point.

The third experiment. A third experiment was planned to test the matter more thoroughly. It was desired to study not only the effect of sulfanilamide on fertility, but to study certain other questions as well. Work by Gunsalus et al. (4) had indicated that first ejaculates contained a higher concentration of bacteria than did second ejaculates collected a few minutes later. Mercier et al. (6) found that first ejaculates were more concentrated, but were of lower volume and contained spermatozoa of which a somewhat smaller percentage were motile. As far as was known, no controlled investigation had been conducted to determine whether first ejaculates were less fertile than second ones, though such an opinion was known to be held by some workers in this field. If such opinions were found to be based upon fact, the question arose as to whether sulfanilamide, supposedly by bacteriostatic action, would eliminate the difference between first and second ejaculates.

The third experiment was conducted during June, 1946, and 16 bulls, 12 Holsteins and 4 Guernseys, were used. The bulls within a breed were paired at random. Two collections, spaced on the average 14 days apart, were made from each bull. At each collection two consecutive ejaculates of semen were obtained and used separately. For any one bull at each collection period one or the other of the ejaculates was diluted with the diluent containing 300 mg. of sulfanilamide per 100 ml. At the next collection period either the first or second ejaculate, whichever had not been treated with sulfanilamide the previous time, received it. The design was thus a $2 \times 2 \times 2$ replicated 8 times and is illustrated thus:

Design of the third experiment

Ejaculate no.	Collection	
	1	2
Bull 1		
1	Y-C-S*	Y-C†
2	Y-C	Y-C-S
Bull 2		
1	Y-C	Y-C-S
2	Y-C-S	Y-C

* Y-C-S = Yolk-citrate-sulfanilamide.
† Y-C = Yolk-citrate.

In order to record the identity of each ejaculate, only one of each collection could be shipped to a particular inseminator. Thus, the number of inseminating units to which semen was shipped (76 different circuits) was divided into two approximately equal groups. For example, at one collection one group of circuits received one ejaculate, the second group the other ejaculate. At the next collection the same circuits received the same ejaculate number but the treatment was different. This design involved the willful confounding of the groups of circuits to which the semen was shipped, with a comparison of first and second ejaculates. This fact did not invalidate the test of whether or not first ejaculates responded differently to sulfanilamide than did second ejaculates, for the design was orthogonal in this respect.

The citrate-sulfanilamide buffer was prepared by bringing a measured

TABLE 3

Average semen quality characteristics and fertility results in the third experiment

Semen character	Diluent used			
	Yolk-citrate		Yolk-citrate-sulfanilamide	
	Ejac. 1	*Ejac.* 2	*Ejac.* 1	*Ejac.* 2
Volume, ml.	5.30	5.78	5.38	5.97
Concentration 1,000's/mm.^{3}	1,397	1,178	1,409	1,188
Motility, %	71.9	73.8	70.6	72.2
Methylene blue reduction time, min.	4.2	5.1	4.3	5.3
Fertility				
Services, no.	1,392	1,092	1,149	1,151
5-mo. N.R., no.	821	661	716	761
5-mo. N.R., %	59.0	60.5	62.3	66.1
Combined				
Services, no.	2,484		2,300	
5-mo. N.R.,* no.	1,482		1,477	
5-mo. N.R., %	59.7		64.2	

* N.R. = non-returns to service.

quantity of water distilled in glass to a boil and adding the predetermined quantity of citrate and of sulfanilamide. The solution immediately was removed from the heat source, poured directly into sterile bottles, and stored in the dark.

The data on semen quality and results of insemination are shown in table 3. The data are arranged to give a comparison of first and second ejaculates and to show the effect of diluents on the fertility of the first and the second ejaculates. If one were interested only in a semen-quality comparison of first and second ejaculates before treatment in this study, the proper comparison is between first ejaculates for yolk-citrate and the second ejaculates for yolk-citrate-sulfanilamide. The other consecutive paired ejaculates are the first for yolk-citrate-sulfanilamide and the second for yolk-citrate.

Statistical analysis of the semen quality characteristics showed that the bulls differed significantly only in volume of semen produced. The specification that two consecutive ejaculates which were satisfactory for use be collected at each of two collection periods was responsible for this result. No semen sample was considered acceptable which contained less than 800,-000 spermatozoa per mm.[3], or which contained less than 60 per cent motile spermatozoa. A total of 20 bulls was sampled for this experiment, 4 of which failed to meet the above specifications.

The spermatozoa of the second ejaculate were significantly more motile than those of the first ejaculate. Highly significant differences were found between first and second ejaculates in concentration and methylene blue reduction time. The first ejaculates were somewhat more concentrated and reduced methylene blue faster than did the second ejaculates. Other differences, as between collection periods, were not of important magnitude and were not mathematically significant.

It should be mentioned that the insemination data were recorded separately for cows being bred for the first time and those which had failed to conceive on first service and were being returned for a second service. The same procedure was followed for the two earlier experiments discussed. In the first two experiments the number of observations was too small to determine whether or not there was a real difference in fertility level for these two groups of cows. In the third, where more observations were made, the difference found was only 1.0 percentage unit, but this small difference was statistically significant. However, the data showed that the addition of sulfanilamide to the diluent increased fertility the same average amount in both groups of cows. In each experiment an analysis of covariance was made on the length of time after collection semen diluted with each diluent was used for insemination. No significant differences were found. For the three experiments combined, 1 per cent of the inseminations were made on the first day (the day of collection), 71 per cent on the second day, 24 per cent on the third, 3 per cent on the fourth, and 1 per cent of all inseminations on the fifth day or later.

As can be seen in table 3 the difference in favor of sulfanilamide in this experiment amounted to a total of 4.5 percentage units. That is, of the 2,300 cows bred with the semen to which sulfanilamide was added, 4.5 per cent more cows apparently conceived than was the case with the 2,484 cows which were bred with the normal yolk-citrate diluent. Statistical analysis of covariance indicated that this difference was greater than was required to show significance at the 1.0 per cent level of probability. Thus, it is concluded that sulfanilamide added to the diluent does increase significantly the probability that a cow will conceive from artificial insemination.

In spite of the fact that no significant differences in semen quality were found between bulls, the differences in fertility among the bulls used was highly significant. However, there was no evidence to indicate that the semen of the several bulls reacted differently to the treatments employed, even though it is known that bacterial contamination varies exceedingly from bull to bull. This fact suggests that the effects of sulfanilamide are largely through the metabolism of semen, and that all normal bull semen would tend to react similarly.

Though first ejaculates tended to be slightly lower in fertility than second ones, an observation bearing out popular belief, the difference observed was not large enough to be mathematically significant when subjected to a test of significance. To test this item effectively a carefully planned and larger experiment should be conducted. The averages given in table 3 suggest that first ejaculates responded less to sulfanilamide treatment that did second ejaculates. However, the sum of squares for error of estimate for the interaction was slightly smaller than the error term, which result forces the authors to conclude that the response to sulfanilamide was not proved different between first and second ejaculates. This observation strengthens the interpretation that the effects of sulfanilamide are largely metabolic ones, for if the effects were due to bacterial control alone one might expect the fertility of the first ejaculates, which contained more bacteria, to be increased more than second ejaculates.

DISCUSSION

It should be mentioned that none of the bulls used in these investigations were known to produce semen containing *Pseudomonas aeruginosa.* This bacterial species is difficult to control and is known to reduce fertility of semen in which it is found as the predominant type (3, 4). Though it was not possible to examine each of the semen samples used in the investigations reported here, the semen of the bulls used had been examined from time to time for bacterial numbers and types, and *Pseudomonas aeruginosa* had not been found.

Reports of the teratogenic effects of sulfonamides when brought into direct contact with certain of the lower animals in the early stages of their

development have appeared in the literature (1, 2, 11, 12). While the authors have not examined each calf produced by the sulfanilamide-treated semen, a number of such animals have been examined and no abnormalities observed. Neither have reports of the birth of abnormal calves been received. When one considers the fact that each milliliter would contain only 3.0 mg. of sulfanilamide, such an amount inseminated into the uterus probably would be absorbed quickly and little would find its way to the developing egg.

The practical value of the results of these studies in routine artificial insemination is obvious. However, in the storage of diluents containing sulfanilamide, care must be taken to keep them out of the light. Preferably, the buffer should be made fresh. However, it is difficult to do so and in large operations this may be a nuisance. Sulfanilamide goes into solution slowly. The authors believe that the simplest way to make the diluent is to bring a required volume of properly distilled water to a boil, add the required amounts of both sodium citrate and sulfanilamide, remove the source of heat, and, when the material has been dissolved and thoroughly mixed, pour the buffer thus prepared into sterile, dark bottles and store it in the dark until used. Buffers so prepared have been used for 2 weeks with no observable influence on their effectiveness. How much longer they can be used satisfactorily has not been determined.

SUMMARY

Three separate experiments involving a total of 8,498 inseminations were conducted to determine the effect on fertility of bull semen of adding sulfanilamide to the yolk-citrate diluent at the rate of 300 mg. per 100 ml. In the first experiment no benefit was observed. In the next two investigations, where the citrate buffer containing sulfanilamide was protected from direct light rays, an increase in fertility by use of the sulfanilamide was obtained. This improvement amounted to 6.1 per cent of the cows inseminated in the second experiment and 4.5 per cent in the third.

The sulfanilamide appeared to influence all semen samples in the same direction, for, in the third experiment, no significant interactions were observed between the treatments and either bulls or first and second ejaculates. These results are interpreted as indicating that the beneficial effects of sulfanilamide on fertility largely are metabolic ones, rather than due to bacterial control alone.

REFERENCES

(1) ANCEL, P., AND LALLEMAND, SUZANNE. Comparison of the Teratogenic Properties of Some Sulfonamides. Arch. de Phys. Biol., 16: 5, supplement 43–45. 1943. (Chem. Abs., 40: 1227. 1946.)

(2) ANCEL, P., AND LALLEMAND, SUZANNE. Mechanisms Put Into Action by Chemoteratogenesis in Higher Vertebrates. Compt. Rend., 219: 143–144. 1944. (Chem. Abs., 40: 1593. 1946.)

(3) GUNSALUS, I. C., CAMPBELL, J. J. R., BECK, G. H., AND SALISBURY, G. W. The Bacteriology of Bull Semen. II. The Effect of Bacteria upon Rapid Tests for Semen Quality. Jour. Dairy Sci., 27: 357–364. 1944.

(4) GUNSALUS, I. C., SALISBURY, G. W., AND WILLETT, E. L. The Bacteriology of Bull Semen. Jour. Dairy Sci., 24: 911–919. 1941.

(5) KNODT, C. B., AND SALISBURY, G. W. The Effect of Sulfanilamide upon the Livability and Metabolism of Bovine Spermatozoa. Jour. Dairy Sci., 29: 285–291. 1946.

(6) MERCIER, E., AND SALISBURY, G. W. The Effects of Season on the Spermatogenic Activity and Fertility of Dairy Bulls Used in Artificial Insemination. Cornell Vet., 36: 301–311. 1946.

(7) SALISBURY, G. W. Fertility of Bull Semen Diluted at 1:100. Jour. Dairy Sci., 29: 695–697. 1946.

(8) SALISBURY, G. W. The Glycolysis, Livability, and Fertility of Bovine Spermatozoa as Influenced by Their Concentration. The Problem of Fertility. Edited by Earl T. Engle. Princeton University Press, Princeton, New Jersey. pp. 134–152. 1946.

(9) SALISBURY, G. W., WILLETT, E. L., AND GUNSALUS, I. C. Some Problems of Bull Semen Storage. Amer. Soc. Anim. Prod. Proc., 32: 210–212. 1939.

(10) SALISBURY, G. W., AND VAN DEMARK, N. L. Stimulation of Livability and Glycolysis by Additions of Glucose to the Egg Yolk-citrate Diluent for Ejaculated Bovine Spermatozoa. Amer. Jour. Physiol., 143: 692–697. 1945.

(11) STOLL, ROLAND. Influence of p-aminobenzenesulfamide on the Development of the Egg, the Embryo, and the Tadpole of Rana fusca. Arch. de Phys. Biol., 16: 136–137. 1943. (Chem. Abs., 39: 4698. 1945.)

(12) STOLL, ROLAND. Influence of Certain Sulfonamides on the Development of the Down of the Chick Embryo. Arch. de Phys. Biol., 16: 5, supplement 59–61. 1943. (Chem. Abs., 40: 2894. 1946.)

THE EFFECT OF REFRIGERATOR STORAGE ON THE KEEPING QUALITIES OF PASTEURIZED MILK

L. H. BURGWALD AND D. V. JOSEPHSON

Department of Dairy Technology, Ohio State University, Columbus

Considerable attention has been directed recently to the problem of the keeping quality of milk in the household refrigerator. The economy of alternate-day or 3-day-a-week delivery of milk was amply demonstrated during the war years with the result that most distributors would prefer to retain this practice. On the other hand, some public health officials have questioned the advisability of maintaining this system of milk distribution because of the highly perishable nature of the product. Under the 3-day-a-week system of delivery the product is consumed in from 1 to 4 days after pasteurization. Therefore, to justify such a practice, we must assure the consumer that the product delivered will retain its initial high qualities for at least 4 days.

Several investigators have demonstrated that good quality, commercially processed milk can be held in a household refrigerator for a considerable period of time without impairment to its nutritional qualities or bacteriological safety.

Nicholas and Anderson (8) studied the keeping qualities of regular pasteurized, pasteurized-homogenized, and raw milks under household refrigerator conditions (40° F.). They found that pasteurized milks could be stored at 40° F. for a period of 2 weeks or more before spoilage would occur. Furthermore, milks that were removed from the refrigerator each day, shaken, and allowed to stand at room temperature for 1 hour retained high quality for approximately 10 days. These investigators based their conclusions on the results of standard plate counts, titratable acidity, and flavor observation, but did not take into account the psychrophilic or coliform organisms. Since the standard plate counts at the time of souring of most of the milks used in their studies were very little different from those of the original fresh milk, it would appear that a consideration of psychrophilic organisms would have been helpful in interpreting their data.

Mott and Mayer (7) conducted a study involving the collection of retail milks in Boston. They found that the standard plate count of grades A and B pasteurized milks increased after the samples were stored at 40° F., and had average counts of 1,300,000 and 1,700,000, respectively, after a storage period of 5 days. Twenty-two samples of certified-pasteurized milk had an average count of 770 per milliliter after 5 days' storage at 40° F.

A far more comprehensive study was reported by Dahlberg (3), who obtained samples from six milk plants in the New York metropolitan area

and stored them at various temperatures. The freshly pasteurized milks had an initial, arithmetic average plate count of about 12,000 colonies per milliliter. Allowing these milks to stand at room temperature for 6 hours had the immediate effect of increasing the plate count slightly and giving more positive coliform tests than were observed with samples not exposed to room temperature. Storage temperatures of 35–40° F. decreased the standard plate count of the milk, while the coliform bacteria remained constant. Samples stored at 45–50° F. showed reduced standard plate counts after 1 day, but considerably higher counts after 4 days. At a storage temperature of 55–60° F. a slight decrease in the standard plate count was observed after 1 day, but thereafter the count increased rapidly. All samples in the group were sour after 4 days. With the exception of this latter group, all samples exhibited good flavor after 7 days' storage. Dahlberg concludes that to insure good keeping quality, milk should not be stored at temperatures above 50° F.

Weber (9) reported an analysis of 10,000 coliform tests collected over a 3-year period for three health departments. These data showed that 21.5 per cent of all pasteurized milks tested were positive to the coliform test during the period of January, February, and March, while 48.0 per cent of the tests were positive during July, August, and September. The higher incidence of coliform-positive milk during the warm months was attributed primarily to a greater degree of contamination of the milk from equipment which is exposed to flies, insects, and air-borne sources.

Dahlberg (4) demonstrated more rapid development of coliform bacteria in refrigerated milk during the summer than during the month of October.

A preliminary report (2) covering certain phases of the present study showed that most commercially pasteurized milks could be held for at least 7 days during the summer months if refrigerator temperatures were held at 45° F. or lower.

The present study was undertaken in order to obtain a more complete picture of the changes that take place during the refrigerator storage of commercially processed market milks, under both summer and winter conditions. It was felt that much of the work reported previously was far too narrow in scope and did not present a complete picture of the bacteriological changes characteristic of stored milk.

METHODS

Two separate studies were undertaken, one under summer conditions (July and August) and the other during the winter (February and March). In each study eight different lots of milk were collected from five different commercial plants immediately after the bottling operation. One-half pint and quart samples of the same milk were collected, and where both homogenized and regular milks were available, a set of each was studied. Samples

were iced and transported to the University laboratories, where exposure treatments, bacterial analyses, vitamin analyses, acidity tests, and flavor observations were conducted prior to storage.

As a standard control, one-half pint samples were stored under the cold unit of a large household-type refrigerator. A sufficient number of these samples was stored so that a different bottle could be removed for analysis and observation at each interval of the storage period.

Of the replicate quart samples, one was placed in the refrigerator immediately, a second was allowed to stand at room temperature (summer, 81–89° F.; winter, 74–79° F.) for 1 hour prior to storage, and a third was exposed to room temperature for 2 hours before refrigeration. All quart samples were placed in the refrigerator on the side away from the cold unit. A careful study of temperature variations was made, using recording and maximum-minimum thermometers. The one-half pint samples stored under the cold unit were held at temperatures ranging from 36–42° F. (2.2–5.5° C.) during the summer study and 37–39° F. (2.8–4° C.) during the winter study. The temperature of storage for quart samples held on the side away from the cold unit ranged from 41–45° F. (5–7.2° C.) during the summer study and 39–42° F. (4–5.5° C.) during the winter study. Therefore, the temperature differential between the samples at the two positions of storage was about 3–5° F. during the summer and about 2–3° F. during the winter.

When quart samples were removed for periodic analyses, they were exposed to room temperature for 5 to 7 minutes before being returned to the refrigerator. During this time the temperature of the samples increased about 2–4° F. in the summertime and about 2° F. in winter.

Bacteriological, vitamin, acidity and flavor analyses were made daily for the first 4 days of storage and then at 7 and 10 days. After 10 days, analyses were run every other day until samples showed evidence of acidity change. At this point, samples were examined daily until 0.03 per cent acid had developed. Samples in which 0.03 per cent acid had developed were considered to be "sour," although the flavor of the product was usually quite good and definitely not sour to the taste.

The standard plate count at 37° C. (98.6° F.) (1) was employed for the detection of mesophilic organisms. Dilutions of 1:100 and 1:1000 were employed at the beginning of storage and gradually increased as storage progressed and counts increased. Psychrophilic organisms were similarly determined with standard tryptone-glucose-extract-milk agar plates incubated at 8–10° C. (46.4–50° F.) for 10 days. One milliliter and 0.1 ml. samples were plated for the first few days of storage, after which time high counts necessitated considerable sample dilution.

Coliform organisms were determined on desoxycholate agar plates incubated at 37° C. (98.6° F.) for 24 hours prior to counting; a 5 ml. sample was plated, using one plate with 2 ml. and one with 3 ml. When counts became high, smaller amounts or dilutions were plated.

Acid development was measured by the standard titration technique using phenolphthalein as the indicator and is expressed as lactic acid.

Riboflavin analyses were conducted according to the method of Hand (5), using a Coleman photofluorometer for measuring fluorescence. Ascorbic acid was determined by the conventional titration procedure employing sodium 2,6-dichlorobenzenoneindophenol dye.

RESULTS

Trend of Bacterial Development during Storage

The regular pasteurized milk usually displayed somewhat better keeping qualities than the homogenized product from the same plant. For the first 7 days of storage the differences usually were insignificant, but thereafter the homogenized milks appeared to support the growth of mesophilic and psychrophilic bacteria better than the unhomogenized product. The type of growth and growth curves were very similar. It was considered justifiable to average the counts of both types of similarly treated milk for the first 7 days of storage. Also one plant participating in the study produced only homogenized milk, while another sold only regular unhomogenized milk.

Since most milk is consumed before it is 7 days old, it seemed unnecessary to make a complete tabulation of bacteriological data taken after this time. Therefore, a geometric mean average was tabulated for each class of samples which had been subjected to the same treatment during the same season.

A graphic presentation of bacteriological data is shown in figure 1. It is very evident that all types of bacterial development were more rapid during the summer season. One factor which undoubtedly contributed to this condition was the slightly higher temperatures that prevailed in the refrigerator during the summer months. Another factor which may have influenced this trend was the greater "temperature shock" given quart samples during the warm summer months both before storage and on days when analytical samples were removed. A difference in the type of microflora present in summer and winter milks also might have contributed to this difference.

These data demonstrate the fact that any treatment which allows the milk to warm up will be reflected in the rate of development of all types of bacteria. This is especially evident from the data taken during the summer months. Furthermore, it can be seen that the more extensive the period of warming before storage, the poorer was the bacteriological keeping quality.

For the most part, mesophilic counts went down or remained rather constant for the first few days of storage, after which their rate of development increased according to the degree of exposure to room temperature (samples 2, 3, and 4). In summer milks which were subjected to warming either before storage or on days when samples were analyzed, the mesophilic count did not show any evidence of increasing until after 2 days of storage.
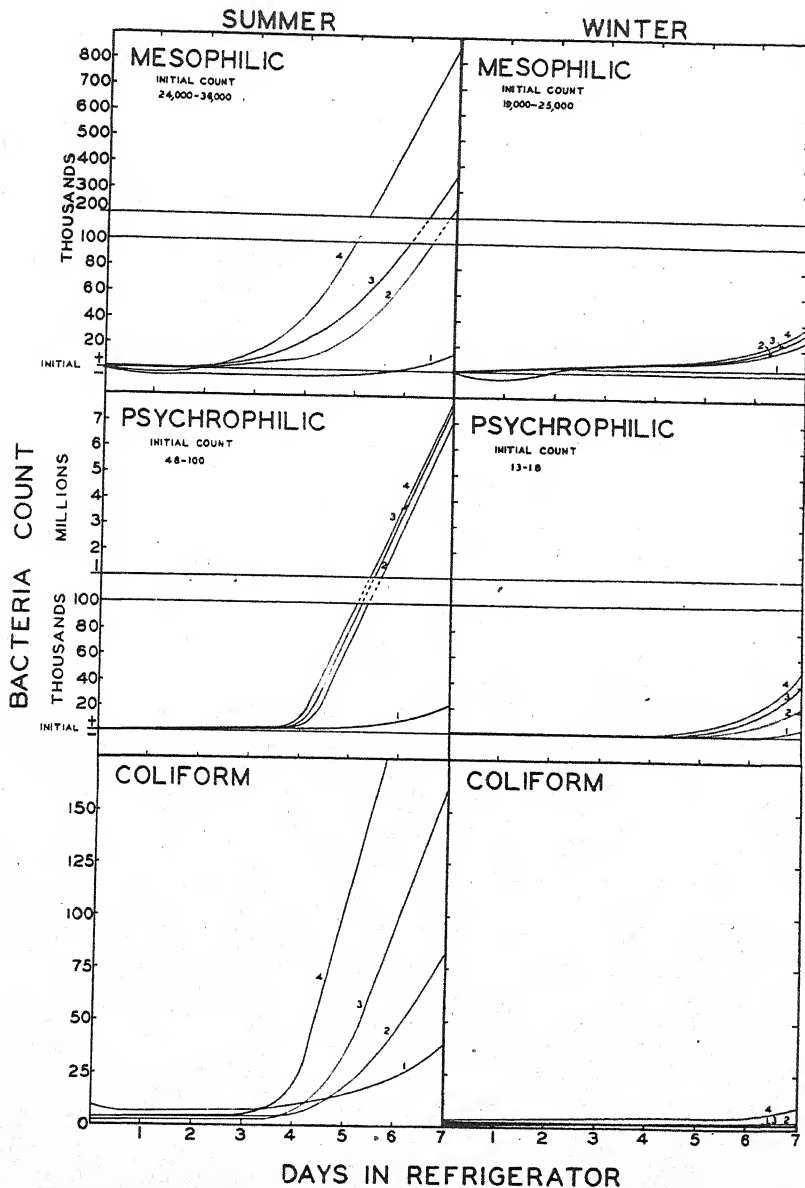
Fig. 1. The effect of refrigerator storage on the micro-flora of commercial market milks (geometric mean averages of all data taken). (1 = one-half pints left in refrigerator continuously; 2 = quarts removed from refrigerator each day for sampling; 3 = quarts allowed to stand out for 1 hour prior to storage, also removed each day for sampling; 4 = quarts allowed to stand out for 2 hours prior to storage, also removed from refrigerator each day for sampling.)

Undisturbed one-half pint samples (sample 1) showed very little change during 7 days of storage. All samples in the winter study, regardless of treatment, showed very little change during the first 7 days of storage, although the effect of warming before storage was becoming evident in samples 2, 3, and 4 at the end of this period.

The development of the psychrophilic organisms followed a similar pattern, but in the summer series their numbers far exceeded those in the mesophilic class after 6 days of storage. These organisms did not increase appreciably during the first 4 days of storage, but after this time they multiplied at a rather rapid rate. In the winter study the psychrophilic counts did not become significant until after 7 days of storage.

Possibly some mesophilic types may be facultative in the sense that they develop psychrophilic activity after adaptation to prolonged low temperature storage. Support for this view may be found in the fact that mesophilic counts frequently decrease during the first few days of storage. Actually these organisms may be going through a temperature adaptation period which eventually brings them into the active and rapidly growing psychrophilic group. Further support for this possibility is the fact that initial psychrophilic counts of the fresh pasteurized milks in this study usually were very low (0–30). If all growth at low temperatures were due to these few true psychrophiles, probably this growth would develop on a more uniform curve. Since rapid growth of psychrophiles does not occur until after 4 days of storage, probably so-called facultative types may supplement the true psychrophilic population in the final rapid deterioration of the milk.

The development of the coliform organisms followed a pattern similar to that of the mesophiles and psychrophiles. As previously observed by Weber (9), the initial coliform counts in summer milks exceeded those of winter milks. Only two samples were found to be free from coliform bacteria and seven samples showed less than one coliform organism per milliliter during the summer trials. All samples were coliform positive at the conclusion of this series or when 0.03 per cent acid developed. On the other hand, six samples were found to be coliform free and nine had less than one organism per milliliter at the beginning of the winter study. Fourteen samples showed no coliform bacteria at the termination of these trials. In the overall tabulation (fig. 1) the coliform count in winter milk was little changed during the first 7 days of storage.

In evaluating these experimental data in terms of the practical keeping problem in home refrigerators, the conclusion seems justified that good quality commercially processed and distributed milks should maintain desirable bacteriological qualities for at least 4 days when stored at 40° F. Under winter conditions bacterial changes are insignificant from a quality standpoint until after 6 or 7 days of storage. In determining the bacteriological quality of stored milk, the psychrophilic organisms must be taken into ac-

TABLE 1

The comparative micro-flora of freshly pasteurized milk and the same milk after storage and development of 0.03 per cent lactic acid
(random samples)

No.	Season of year	Type of bottle	Treatment*	Bacteria counts				Total storage period (days)
				Mesophilic		Psychrophilic		
				Fresh	After storage	Fresh	After storage	
1	Summer	½ pint	A	290,000	38,000	13	177,500,000	21
2	"	½ pint	A	9,000	530,000	8	167,000,000	24
3	"	½ pint	A	61,000	83,000	21	131,400,000	25
4	"	Quart	B	13,600	5,800,000	9	270,000,000	13
5	"	Quart	C	12,200	3,500,000	8	190,000,000	13
6	"	Quart	C	292,000	162,000	26	189,000,000	14
7	"	Quart	D	315,000	221,000	23	178,000,000	13
8	Winter	½ pint	A	14,900	19,600	74	164,000,000	33
9	"	½ pint	A	94,000	213,000	4	248,000,000	35
10	"	Quart	B	18,400	42,000	24	328,000,000	23
11	"	Quart	B	5,000	165,000	1	277,000,000	24
12	"	Quart	C	18,600	21,900	31	315,000,000	23
13	"	Quart	C	67,000	1,730,000	270	150,000,000	12
14	"	Quart	D	128,000	1,650,000	5	260,000,000	20
15	"	Quart	D	39,000	450,000	<1	201,000,000	17

* A = One-half pint—left in refrigerator continuously; B = Quart—removed from refrigerator each day for sampling; C = Quart—allowed to stand out for 1 hour prior to storage, also removed from refrigerator each day for sampling; D = Quart—allowed to stand out for 2 hours prior to storage, also removed from refrigerator each day for sampling.

count because of their relatively greater numbers after 5 or 6 days at storage temperatures.

Changes in Micro-Flora during Storage

In analyzing the data taken in both the summer and winter studies and attempting to correlate the initial and final bacterial counts with the relative keeping quality of a product, nothing could be established as an index for a potential trend in bacterial growth. The data presented in table 1 represent a random selection of samples from these studies. These data demonstrate the fact that the initial mesophilic count of the freshly pasteurized milk is not a dependable index of the keeping quality of the product from a bacteriological standpoint. The initial psychrophilic count does not serve as a basis upon which to predict the potential keeping quality of any type of milk, regardless of treatment.

By comparing the data for samples no. 1 and 2 in the summer study and 8 and 9 in the winter study, it can be seen that high count milks (nos. 1 and 9) can have keeping qualities as acceptable as similar milks (nos. 2 and 8) with relatively low initial counts. This should not be taken to imply that the standard plate count is not a useful test for evaluating general milk quality, but it does suggest that its use as an index of potential keeping quality of pasteurized milk is questionable and probably unsound.

Acid Development in Stored Milks

The length of the storage period which elapsed before milks developed 0.03 per cent acid (expressed as lactic acid) naturally varied among the different lots and treatments. One-half pint samples which remained undisturbed in storage did not develop 0.03 per cent lactic acid until 21.8 days (average) of storage in the summer study and 26.6 days (average) in the winter trials. Quart samples which were allowed to stand out at room temperature for 2 hours before storage and were further exposed at periodic sampling periods developed 0.03 per cent lactic acid in 12.4 days (average) in summer and 16.6 days (average) in winter trials.

Most samples examined in these studies showed evidence of lactic acid development while mesophilic counts (standard plate) were still quite low. Some samples developed 0.03 per cent lactic acid while exhibiting a mesophilic count lower than that of the original fresh milk. Nicholas and Anderson (8) have reported "sour" flavor in samples with mesophilic counts which were little different from those of the fresh products.

In the present study the psychrophilic organisms largely are responsible for the development of the lactic acid in milk held in refrigerator storage at 40° F. The data plotted in figure 2 are for three samples, chosen at random from the many which were plotted and compared. A close correlation exists between the growth of psychrophilic bacteria and the development of lactic acid. By comparison, the magnitude of change in the numbers of mesophilic

organisms is relatively insignificant. This would suggest that the acid developed, at least in the early stages of deterioration, is a product of the psychrophilic bacteria. Apparently consideration of psychrophiles is of paramount importance in studies of this kind, particularly since acid development is considered to be one of the primary factors in milk spoilage or deterioration.

FIG. 2. The relationship between lactic acid development and bacterial growth in milks refrigerated for long periods of time (random samples from different periods during study).

The Effect of Refrigerator Storage on Riboflavin and Ascorbic Acid

Since riboflavin and ascorbic acid are subject to destruction by exposure to light and the latter is subject to natural oxidation on storage, a study of these two vitamins in stored milks appeared desirable.

The data shown in table 2 demonstrate the stability of riboflavin and ascorbic acid in milks stored under household refrigerator conditions. Because of the absence of light in the refrigerators, it is not surprising that riboflavin was stable in samples held continuously under these conditions. Even the quart samples B, C and D, which were exposed to the light of the room at the time of periodic samplings, still retained practically all of their riboflavin throughout the extended storage period. Apparently the normal daylight present in the laboratory was insufficient to cause any great degree of photolysis of this vitamin during these periodic exposures. These results confirmed our previous observations (6) where samples exposed in a bright kitchen for 2 hours lost only a small percentage of riboflavin.

TABLE 2

The effect of refrigerator storage on the stability of riboflavin and ascorbic acid (random samples)

No.	Season of year	Type of bottle	Treatment*	Ascorbic acid mg./l.			Riboflavin mg./l.		Total storage period (days)
				Fresh	After 1 day	After 4 days	Fresh	After storage	
1	Summer	½ pint	A†	7.1	1.6	0.0	1.51	1.52	28
2	"	½ pint	A	8.3	3.2	0.0	1.62	1.61	31
3	"	Quart	B	11.3	9.7	2.0	1.57	1.56	13
4	"	Quart	C†	14.0	13.5	12.1	1.61	1.61	14
5	"	Quart	D	10.1	8.1	2.8	1.55	1.52	12
6	Winter	½ pint	A†	9.1	2.8	0.0	1.62	1.63	25
7	"	½ pint	A†	15.1	12.7	12.0	1.73	1.71	24
8	"	Quart	B	9.1	0.0	0.0	1.73	1.71	15
9	"	Quart	C	6.7	2.0	0.0	1.39	1.37	20
10	"	Quart	D	6.6	1.9	0.0	1.40	1.39	20

* A = One-half pint—left in refrigerator continuously; B = Quart—removed from refrigerator each day for sampling; C = Quart—allowed to stand out for 1 hour prior to storage, also removed from refrigerator each day for sampling; D = Quart—allowed to stand out for 2 hours prior to storage, also removed from refrigerator each day for sampling.
† Homogenized milk.

The retention of ascorbic acid during storage was very poor in most samples. Even the one-half pint samples which were refrigerated continuously and not exposed to light retained very little ascorbic acid after 1 day in storage. The exposure of samples to room temperature and light conditions for 2 hours caused an initial loss of about 20 per cent of this vitamin during the summer studies and about 12 per cent in the winter trials.

The high level of ascorbic acid found in the homogenized milk from one plant (samples 4 and 7, table 2) was difficult to explain. This milk was pasteurized at 170–172° F. for 16 seconds. Additional samples procured over a period of weeks showed that this heat treatment produced some sulphydryl compounds which protected ascorbic acid. Whereas most samples (homogenized or unhomogenized) were completely depleted of ascorbic acid within 4 to 6 days, most of these homogenized samples retained significant quantities of this vitamin throughout the entire storage period. The regular unhomogenized milk from this plant was pasteurized at a conventional temperature and ascorbic acid losses conformed with those experienced with milk from other plants.

Flavor Changes during Storage

During the winter months the samples in these studies exhibited somewhat better keeping qualities than did those in summer months. However, off-flavor development was somewhat more prevalent in the winter. Oxidized flavor was more frequently found during the winter trials than during the summer. Only one sample developed a typical oxidized flavor during the summer trials.

On the whole, the milks retained their initial fine flavor until very near the end of the storage period (0.03 per cent developed acid), when stale, unclean, acid or bitter flavor defects usually developed. With the exception of the occasionally observed oxidized flavor previously mentioned, practically all samples retained their initial flavors for at least 7 days. In several cases samples exhibited excellent flavor for more than 20 days of storage. Apparently the problem of flavor development during storage is relatively no more important when milk is consumed within a week after pasteurization than if consumed within 2 days.

DISCUSSION

One of the most interesting observations made in the course of these investigations is the apparent rôle of the psychrophilic bacteria in the deterioration of milk stored under refrigerator conditions. The initial mesophilic count (standard plate) appears to have little bearing on the potential keeping qualities of milks stored under the conditions of these experiments. Mesophilic counts at the termination of the storage period (0.03 per cent developed acid) frequently were little different from those exhibited by the

fresh milk before storage. The suggestion that some mesophiles become adapted to storage conditions and eventually grow at these low temperatures seems justified, since mesophilic counts usually go down during the first few days of storage. This is further supported by the fact that rapid psychrophilic growth does not start until after 4 or 5 days of storage. The initial psychrophilic count does not appear to be a dependable index for predicting the potential keeping qualities of milk. The number of psychrophilic organisms in fresh pasteurized milk usually was found to be low (0–30 per ml.), but a considerably higher count never was found to prevent the product from exhibiting excellent keeping qualities.

The development of acidity during storage was found to correlate quite definitely with psychrophilic growth. The mesophilic populations present were comparatively small and probably contributed very little to the development of lactic acid under the conditions of these experiments.

The general growth curves for mesophilic, psychrophilic, and coliform organisms all followed like patterns. The magnitude of population change was much greater in the case of the psychrophiles and was by far the least for the coliforms. In the summer studies little change was observed in any of the bacterial counts until after 4 days of storage, while in winter important changes were not noted until after 7 days.

The flavor of the milks changed very little until near the end of the storage period (0.03 per cent developed acid), when stale, unclean, acid or bitter flavors usually developed. In several cases during the winter studies and in one case during the summer trials, the oxidized flavor developed soon after the milk went into storage. With these exceptions the flavor of the milks was exceptionally good until measureable lactic acid was produced. In very few cases was it possible to taste any evidence of acid until the concentration was in excess of 0.03 per cent.

The storage of milk in the dark at 40° F. did not change the riboflavin content of the product, and the periodic exposure of milk to the daylight in the laboratory had little or no effect upon this vitamin. Ascorbic acid was depleted very rapidly both before and after storage, only insignificant amounts of this vitamin usually remaining after 1 day of storage. One exception was found in the case of homogenized milk which had been flash pasteurized at 170–172° F. The protective effect of sulphydryl compounds produced by this pasteurization temperature accounted for the high levels of ascorbic acid observed in and retained by these samples in storage.

Good quality pasteurized market milks will maintain good bacteriological and flavor qualities for at least 4 days in summer and 6 to 7 days in winter, if refrigerator temperatures are maintained near 40° F. Exposure of milk to room temperatures naturally will impair the keeping qualities of the product, but with good distribution practices and prompt removal of the milk from the doorstep, milk should retain excellent quality for a considerable period of time.

SUMMARY

The psychrophilic bacteria which develop in milk during refrigerator storage are primarily responsible for the deterioration of the product. These organisms apparently are responsible for the development of the acid which is produced in milk during storage at 40° F.

The initial mesophilic (standard plate) and psychrophilic counts do not serve as an index of the potential keeping quality of milk being stored at about 40° F. Mesophilic counts frequently are little changed during the entire storage period.

Riboflavin in milk is not affected by refrigerator storage, and periodic exposure to room daylight has no noticeable effect.

The ascorbic acid content of milk is depleted rapidly both before and during storage. After 1 day of storage, only insignificant quantities remain in milks processed by conventional procedures.

Milk of good quality can be expected to retain excellent bacteriological and flavor qualities for at least 4 days during the summer months and 6 to 7 days during winter months if refrigerator temperatures are maintained near 40° F.

REFERENCES

(1) AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Dairy Products. 8th Edition. 1941.

(2) BURGWALD, L. H., AND JOSEPHSON, D. V. Keeping Quality of Milk. Amer. Milk Rev., 8: 46. 1946.

(3) DAHLBERG, A. C. The Keeping Quality of Pasteurized Milk in the New York Metropolitan Area during Cool Weather as Determined by Bacterial Counts, Presence of Coliform Bacteria and Flavor. Jour. Dairy Sci., 28: 779. 1945.

(4) DAHLBERG, A. C. The Relationship of the Growth of Bacteria in Pasteurized Milk Held at Refrigeration Temperatures. Jour. Dairy Sci., 29: 651–655. 1946.

(5) HAND, D. B. Determination of Riboflavin in Milk by Photoelectric Fluorescent Measurements. Indus. and Engin. Chem., Analyt. Ed., 11: 306. 1939.

(6) JOSEPHSON, D. V., BURGWALD, L. H., AND STOLTZ, R. B. The Effect of Route Delivery on the Flavor, Riboflavin and Ascorbic Acid Content of Milk. Jour. Dairy Sci., 29: 273–284. 1946.

(7) MOTT, F. E., AND MAYER, H. Deterioration of Milk by Bacterial Growth under Refrigeration at 40° F. Certified Milk, 17(199): 3. 1942.

(8) NICHOLAS, J. E., AND ANDERSON, T. G. Keeping Qualities of Milk. Refrig. Engin., 44: 370. 1942.

(9) WEBER, C. W. Coliform Bacteria in Pasteurized Milk. New York State Assoc. of Milk Sanitarians, Proc., 21: 139–144, 146, 148. 1944.

COMPOSITION OF MARES' MILK AS COMPARED WITH THAT OF OTHER SPECIES[1]

ARTHUR D. HOLMES, ALBERT F. SPELMAN, C. TYSON SMITH, AND JOHN W. KUZMESKI

Massachusetts Agricultural Experiment Station, Amherst

The skeleton of mammals calcifies very rapidly during the period immediately following birth when there is a rapid progressive penetration of calcium salts into the cartilaginous areas of the skeletal tissues. Furthermore, from an extensive review of the literature dealing with the requirement for calcium during growth of the human infant, Holmes (33) showed that the most rapid skeletal growth occurred during the initial period of the life cycle and that this probably is true for other mammals. Nearly 50 years ago Abderhalden (1) reported that the average foal doubles its birth weight in 60 days. During that period the foal subsists very largely on its mother's milk, and obviously all the mineral elements needed by the foal for bone and tissue building must be derived primarily from the mare's milk. However, very few data are available regarding the mineral content of mares' milk. A number of investigators have reported the amount of ash found in mares' milk: *i.e.,* Linton (39) found 0.28–0.95 per cent, with an average of 0.51 per cent; Vieth (67) found 0.26–0.36 per cent, with an average of 0.30 per cent in the milk from 15 milk mares; Hildebrant (24), 0.32–0.74 per cent; Papp (51), summer milk 0.3 per cent and winter milk 0.5 per cent; Dittrich (13), 0.29–0.60 per cent; and Masek (42), 0.35 per cent in the milk of the wild mare Przewalski kept in the zoological gardens in Prague. However, these data supply little or no information concerning the amount of various mineral constituents such as calcium, magnesium, phosphorus, and potassium in mares' milk, and the present study was undertaken to accumulate data regarding these elements.

EXPERIMENTAL

The mares' milk used in this study was produced by one Palomino and four Percheron mares. All the mares were mature, well-developed, normal animals. Their ages varied from 4 to 10 years and, numbered 1 to 5, consecutively, they were in their second, fifth, first, fifth and first lactations, respectively. The study was made in the spring. The mares' daily ration consisted of 3 quarts of crushed oats, five large ears of thoroughly matured dent corn, all the good-quality hay they desired, and as much rapidly-growing grass as they could eat in 3 or 4 hours. The Palomino mare, no. 1, weighed 1,100 lbs. and the Percherons from 1,600 to 1,900 lbs. The stage of lactation varied from the ninth day for the first sample of milk from

mare no. 1 to the 129th day for the last sample from mare no. 5. While the stage of lactation was not the same for all the animals, the days on which the milk was collected were identical for all the mares. The samples of milk were collected as described by Holmes and Lindquist (28) and Holmes and associates (31). Samples were taken directly to the laboratory, thoroughly mixed, and assayed for water, protein, reduced ascorbic acid, phosphorus, potassium, magnesium, and calcium by the official methods of the Association of Official Agricultural Chemists (3). In order to minimize possible decomposition, the ascorbic acid assays were begun within an hour after the milk was obtained from the mares.

COMPARISON OF MARES' MILK WITH THAT OF OTHER SPECIES

The results of the assays of the 26 samples of mares' milk collected during the early lactation period are reported in table 1. Data concerning the composition of milk produced by several mammalian species are included in table 2. These data do not constitute all that are available in this field, but they should be sufficient to permit a comparison of the composition of mares' milk with that of other species.

The composition of the milk from each of the five mares varied somewhat from day to day. Since the values obtained for the Palomino mare's milk were not in close agreement with those obtained for the other mares, the average values for milk from the four Percheron mares have been used in the discussion that follows.

Water. The average values for the water content of the milk examined in this study ranged from 88.7 per cent for mare no. 2 to 90.2 per cent for mare no. 4. These values are in good agreement with the results obtained by the English investigators and are greater than that reported for cow, goat, ewe, buffalo, camel, or human milk. However, the difference in water content between mares' milk and that of other species is not sufficiently large to preclude a satisfactory comparison of the other constituents of the milks of the various species.

Protein. The amount of protein in the milk was quite uniform for the individual mares, but the average values for the different animals varied from 2.0 to 2.7 per cent. The average value of 2.3 per cent obtained in this study is higher than that reported by Vieth (67) and Morrison (44) but lower than the values reported by Linton (39) and Hildebrant (24). The protein content of the mares' milk was less than that of cow, goat, ewe, buffalo, camel, or sow milk, but it was similar to that of human milk, as reported by several investigators. In view of the similarity in protein and lactose content of mare and human milk, attention has been given to the possibility of using mares' milk for infant feeding. From his studies in this field, Frendenberg (20) found that because of its low fat content, mares'

TABLE 1

Composition of mares' milk

Mare no.	Day of lactation	Water (%)	Protein* (%)	Ascorbic acid (mg./l.)	Phosphorus (mg./100 g.)	Potassium (mg./100 g.)	Magnesium (mg./100 g.)	Calcium (mg./100 g.)
1	9	88.2	2.9	62	78	74	10.8	126
	10	89.2	3.3	83	79	87	10.8	122
	11	89.2	3.3	88	85	88	11.8	131
	12	89.1	3.2	90	87	99	11.2	133
	13	89.8	3.3	90	90	91	14.7	107
	14	89.1	3.0	73	79	84	10.6	127
Av.		89.1	3.2	81	83	87	11.7	124
2	12	88.6	2.9	95	76	63	11.2	142
	13	88.4	2.7	94	61	46	9.1	111
	14	88.6	2.5	99	65	53	8.9	122
	15	88.9	2.6	101	71	58	9.7	135
	16	88.8	2.6	101	68	60	9.4	127
Av.		88.7	2.7	98	68	56	9.7	127
3	25	89.9	2.4	79	72	87	12.9	115
	26	89.6	2.5	83	74	86	12.9	112
	27	90.1	2.4	70	70	77	12.0	106
	28	89.9	2.3	73	69	76	9.7	102
	29	89.1	2.2	75	69	71	8.6	97
Av.		90.0	2.4	76	71	79	11.2	106
4	38	90.3	2.1	88	60	65	9.0	98
	39	90.0	2.1	88	59	66	9.3	97
	40	90.3	2.0	74	58	62	8.3	93
	41	90.2	2.1	70	58	56	8.9	94
	42	90.0	2.2	68	59	66	7.3	91
Av.		90.2	2.1	78	59	63	8.6	95
5	125	89.5	2.0	112	52	55	7.1	79
	126	90.1	2.0	103	50	50	6.4	80
	127	89.9	2.0	100	52	55	6.6	82
	128	90.1	1.9	108	52	62	6.2	78
	129	89.6	2.0	104	54	59	5.0	80
Av.		89.8	2.0	105	52	56	6.3	80
Av. Percherons (mares 2 to 5, inc.)		89.7	2.3	89	63	64	9.0	102

* Protein = N × 6.38.

TABLE 2

Comparison of mares' milk with that of other species

Source of milk	Water (%)	Protein (%)	Ascorbic acid (mg./l.)	Ash (%)	Phosphorus (mg./100 g.)	Potassium (mg./100 g.)	Magnesium (mg./100 g.)	Calcium (mg./100 g.)
Mare	89.04 (39) 90.13 (67) 90.70 (34)	1.65 (67) 2.06 (39) 2.55–3.13 (24) 3.00 (44)	27–115 (54) 87–197 (10) 95 (9)	0.51 (39) 0.30 (67) 0.32–0.74 (24) 0.30–0.50 (51) 0.29–0.60 (13) 0.35 (42)	50 (44)	80 (44)		80 (44)
Cow	86.21 (50) 87.90 (2) 87.10 (66)	3.20 (34) 3.13–3.77 (2) 3.50 (44) 3.30 (14) 3.20 (66)	21–22 (61) 18–20 (32) 25 (38) 20–25 (46) 18 (26) 16 (30) 12–15 (42) 16 (29)	0.69 (14) 0.70 (66) 0.72 (50) 0.68–0.74 (34) 0.70 (44)	68–92 (48, 49, 57, 58) 76–113 (11) 82 (37) 84–127 (63) 93 (61) 100 (36)	123–180 (12) 126–192 (52) 105–172 (2) 143 (60) 140 (44)	5–22 (48, 49, 57, 58) 10–12 (52) 11–17 (63) 7–12 (41) 10 (36)	90–155 (12) 94–150 (63) 96–161 (11) 102–148 (2) 111–132 (52) 118 (60) 120 (36) 155–171 (41)
Goat	87.14 (2) 85.71 (34)	4.29 (34) 3.40 (27) 3.70 (44) 2.99–3.71 (52) 3.99 (4)	5–20 (55) 20 (65) 17 (30) 13 (22) 85 (56) 9 (8) 45 (9)	0.31 (70) 0.94 (6) 0.78 (2) 0.81 (25) 0.81 (43) 0.78 (71) 0.79 (5) 0.80 (68)	98 (5) 124 (19) 96 (6) 104 (43) 112 (27) 100 (44)	193 (6) 171–228 (52) 106–242 (27) 150 (44)	14–20 (52) 13–22 (27)	114 (5) 131 (43) 138 (6) 141 (19) 137 (27) 130 (44) 117–150 (52)

TABLE 2 (Continued)

Source of milk	Water	Protein	Ascorbic acid	Ash	Phosphorus	Potassium	Magnesium	Calcium
	(%)	(%)	(mg./l.)	(%)	(mg./100 g.)	(mg./100 g.)	(mg./100 g.)	(mg./100 g.)
Ewe	82.90 (2) 80.82 (34)	6.50 (44) 6.52 (34) 5.44 (2)		0.85 (2) 0.89 (34) 0.90 (44)	120 (44)	190 (44)		210 (44)
Buffalo	76.80 (2) 82.09 (2) 82.69 (34)	5.88 (34) 4.16–6.04 (2)	10 (8)	0.78–0.86 (2) 0.76 (34)				
Camel	87.61 (2)	2.98 (2)		0.70 (2)				
Sow		5.90 (44)		1.00 (44)				
Elephant				0.40 (64)				
Human	87.43 (23) 87.68 (6) 87.41 (34)	2.29 (34) 1.05 (7) 1.63 (23) 1.10 (17) 0.96 (18)	35 (8) 27 (9) 40 (16) 21–90 (53) 5–23 (69) 52 (45) 60–80 (59) 37 (35) 18–46 (62)	0.20 (19) 0.31 (70) 0.31 (6) 0.20 (7) 0.21 (43) 0.18–0.21 (71) 0.31 (34)	13 (43) 20 (19) 16 (47)			23 (19) 30 (47) 35 (43)

Figures in parentheses refer to the number of the reference cited.

milk could not be used successfully for infant feeding unless 1.0 to 1.5 per cent of cows' milk fat was added.

Reduced ascorbic acid. The average ascorbic acid content of the milk produced by the Percheron mares, 89 mg. per liter, was much higher than that previously reported by Holmes *et al.* (31), but was within the range reported by Cimmino (10) and by Rasmussen *et al.* (54), and was practically identical with the value reported by Cimmino (9). The reduced ascorbic acid content of fresh mares' milk was much greater than that of cow, goat, or buffalo milk. The reported ascorbic acid content of human milk varies over wide limits. Widenbaur and Kühner (69) found a minimum of 5 mg. per liter, and Quesada (53) reported as much as 90 mg. When Ingalls *et al.* (35) administered massive doses of ascorbic acid orally or intravenously to women, the milk subsequently secreted had an ascorbic acid content of 116 mg. per liter. A number of investigators (16, 35, 59) have increased the amount of ascorbic acid in human milk several-fold by adding ascorbic acid-rich foods to the diet. Furthermore, other workers found a marked seasonal variation. Sinkko (62) made observations on ten subjects in February and again in September and found 1.8 mg. and 4.6 mg. per liter, respectively. Other authors have found seven or eight times as much ascorbic acid in human milk in late summer as in the winter.

Ash. The reported assays for the amount of ash in mares' milk indicate that the average value is between 0.4 and 0.5 per cent. The mineral content of cow, goat, ewe, buffalo, and camel milk is 0.7 per cent. A number of investigators have reported upon the mineral content of human milk and the average value seems to be between 0.2 and 0.3 per cent. Thus mares' milk contains about two-thirds as much ash as the other species of animals cited and about twice as much as human milk.

Phosphorus. The phosphorus in the twenty samples of milk from the Percheron mares averaged 63 mg. per 100 g. This value is somewhat higher than the value reported by Morrison (44), but it is only about two-thirds as high as in cows' milk. Goats' milk has a slightly higher phosphorus content than cows' milk, but judged by the limited reports found in the literature, human milk contains only about one-fifth as much phosphorus as mares' milk.

Potassium. The average amount of potassium (64 mg. per 100 g.) found in the samples of Percheron mares' milk included in this study is somewhat less than the 80 mg. reported by Morrison (44). The potassium content of cows' milk, as indicated by the reports cited in table 2, is more than twice that of mares' milk. The amount of potassium in goats' and ewes' milk is appreciably higher than that of cows' milk and decidedly higher than that of mares' milk.

Magnesium. The magnesium content of mares' milk as obtained for the individual mares was fairly consistent, but the average values for the differ-

ent animals varied from 6.3 mg. to 11.2 mg. per 100 g. The available literature supplied no data regarding the amount of magnesium in mares' milk. The mean result obtained for the four Percheron mares was 9 mg. of magnesium per 100 g. of milk. This definitely is less than the amount of magnesium present in cows' milk and goats' milk. No data were found concerning the amount of magnesium in human milk.

Calcium. The calcium content of milk, particularly that of cows and goats, has received considerable attention, since these milks are used most frequently as substitutes for human milk in infant feeding. The mares' milk was found to contain an average of 102 mg. of calcium per 100 g. This amount is significantly less than the amount present in cows' or goats' milk. However, it is three or four times the amount reported by Forbes and Keith (19), Nims *et al.* (47), and Maynard (43) for human milk.

Calcium-phosphorus ratio. Since calcium and phosphorus are required in much larger amounts than other minerals for bone formation, the ratio of calcium to phosphorus is of interest to those concerned with animal and human nutrition. The ratio of calcium to phosphorus in cows' milk has been reported as 1.40 (37), 1.20 (36), and 1.27 (60); in goats' milk as 1.44 (6), 1.26 (43), 1.22 (27), 1.14 (19), and 1.16 (5). The values noted in the literature for the ratio of calcium to phosphorus in human milk were very limited and extremely variable: *i.e.*, 2.69 (43), 1.84 (47), and 1.25 (19). The ratio of calcium to phosphorus obtained for mares' milk was 1.62, a value which is definitely higher than that of cows' or goats' milk but possibly lower than the calcium-phosphorus ratio of human milk. It may be noted that Linton (40) has reported that the calcium and phosphorus content of mares' milk decreased linearly with the advance of lactation, but the calcium-phosphorus ratio remained quite constant throughout the lactation period.

SUMMARY

Twenty-six samples of milk produced by one Palomino and four Percheron mares were assayed for water, protein, ascorbic acid, phosphorus, potassium, magnesium, and calcium. The milk was produced in the early lactation period during late winter and early spring months when the mares were subsisting principally upon hay and grain. The milk from the Palomino mare contained more protein, phosphorus, potassium, and magnesium than the Percheron mares' milk. The average values for the milk of the Percheron mares were: water 89.7 per cent, protein 2.3 per cent, reduced ascorbic acid 89 mg. per liter, phosphorus 63 mg., potassium 64 mg., magnesium 9.0 mg., and calcium 102 mg. per 100 g. These values indicate that mares' milk contains more water than cow, goat, ewe, buffalo, camel, or human milk; less protein than cow, goat, ewe, buffalo, or camel milk, but more than human milk; more ascorbic acid than cow, goat, or human milk;

less phosphorus than cow or goat milk but more than human milk; only about one-third as much potassium as cow or goat milk; and less magnesium and calcium than cow or goat milk, but about four times as much calcium as human milk. The ratio of calcium and phosphorus is considerably higher in mares' milk than in cows' or goats' milk but possibly lower than in human milk.

The authors wish to express their thanks to Mr. Richard Nelson of the Animal Husbandry Department for his assistance in collecting the samples of mares' milk and for his interest and cooperation.

REFERENCES

(1) ABDERHALDEN, E. Zurechtstellung. Ztschr. f. Phys. Chem., 27: 594. 1899.

(2) ASSOCIATES OF L. A. ROGERS. Fundamentals of Dairy Science. 2nd Ed. Reinhold Publishing Corp., New York. 1935.

(3) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and Tentative Methods of Analysis. 6th Ed. Washington, D. C. 1945.

(4) BAGNALL, H. H. Goat Milk. Analyst, 68: 148. 1943.

(5) BESLEY, A. K. Recent Investigations of Goat Milk. Amer. Jour. Pub. Health, 30: 182–185. 1940.

(6) BOSWORTH, A. W., AND VAN SLYKE, L. L. The Casein and Salts of Goat's Milk. N. Y. State Agr. Expt. Sta. Tech. Bul. 46. 1915.

(7) BROWN, MINERVA, MACY, ICIE G., NIMS, BETTY, AND HUNSCHER, HELEN A. Human Milk Studies. VIII. A Comparison of the Composition of the Milk from the Two Breasts. Amer. Jour. Dis. Children, 43: 40–51. 1932.

(8) CHAKRABORTY, R. K. The Vitamin C Content of Some Indian Food Materials. Indian Jour. Med. Res., 23: 347–351. 1935. (Chem. Abs., 30: 3101. 1936.)

(9) CIMMINO, A. Vitamin C in Milk of Women, Cows and Other Mammals. Quaderni della Nutr., 5: 239–288. 1938. (Chem. Abs., 33: 3471. 1939.)

(10) CIMMINO, A. Vitamin C in Horse Milk. Ann. d'Ig., 50: 471–472. 1940. (Chem. Abs., 38: 1780. 1944.)

(11) CRANFIELD, H. T., GRIFFITHS, D. G., AND LING, E. R. The Composition of Milk. II. Variation of Percentage of Mineral Constituents in Cow's Milk, and Their Relationship with the Solids not Fat and Protein Content. Jour. Agr. Sci., 17: 72–93. 1927.

(12) CRICHTON, J. A. The Mineral Requirements of the Dairy Cow. Jour. Dairy Res., 2: 1–37. 1930.

(13) DITTRICH, H. The Composition of Mare Milk. Milchw. Forsch., 19: 406. 1938. (Chem. Abs., 33: 2967. 1939.)

(14) ECKLES, C. H. Dairy Cattle and Milk Production. The Macmillan Co., New York. 1939.

(15) ECKLES, C. H., AND SHAW, R. H. The Influence of the Stage of Lactation on the Composition and Properties of Milk. U. S. Dept. Agr., Bur. Anim. Indus., Bul. 155. 1913.

(16) ESCUDERO, A., AND HERRAIZ, MARIA L. Human Milk as a Source of Vitamin C for Infants. Pubs. inst. nacl. nutricion. CPa 5: 192–199. 1942. (Chem. Abs., 39: 1445. 1945.)

(17) ESCUDERO, P., AND PIERANGELI, E. Chemical Composition of Human Milk; Factors which Influence Its Nutritive Value. Pubs. inst. nacl. nutricion. CPa 5: 180–191. 1942. (Chem. Abs., 39: 1445. 1945.)

(18) ESCUDERO, P., AND REPETTO, ISABEL GRACIA. The Chemical Composition of Human Milk Used by the National Institute of Nutrition. Pubs. inst. nacl. nutricion. CNP 27: 79–85. 1944. (Chem. Abs., 39: 4363. 1945.)

(19) FORBES, E. B., AND KEITH, M. Phosphorus Compounds in Animal Metabolism. Ohio Agr. Expt. Sta. Tech. Bul. 5. 1914.

(20) FRENDENBERG, E. Infant Feeding with Mare Milk. Ann. Paediat., 166: 338–364. 1946. (Chem. Abs., 41: 181. 1947.)

(21) FRENZEL, FELIX. Metabolism Experiments with Suckling Foals. II. Milk Utilization by Suckling Foals. Biedermann's Zentbl. f. Agr. Chem., Abt. B, Tierernährung., 9: 316–339. 1937. (Expt. Sta. Record, 79: 234. 1938.)

(22) GAMBLE, J. A., ELLIS, N. R., AND BESLEY, A. K. Composition and Properties of Goat's Milk as Compared with Cow's Milk. U. S. Dept. Agr. Tech. Bul. 671. 1939.

(23) GARDNER, J. A., AND FOX, F. W. On the Variations in the Composition of Normal Human Milk. The Practitioner, 114: 153–168. 1925.

(24) HILDEBRANT, A. Analysis of Mare's Milk. Milchw. Zentbl., 46: 273, 289, 305, 317. 1917. (Chem. Abs., 12: 1480. 1918.)

(25) HOLM, G. E., AND WEBB, B. H. Buffer Capacities of Various Milks and Proprietary Infant Foods. Amer. Jour. Dis. Children, 40: 260–268. 1930.

(26) HOLMES, A. D., JONES, C. P., WERTZ, ANNE W., ESSELEN, KATHERINE, AND McKEY, BEULA V. The Ratio of Ascorbic, Nicotinic, and Pantothenic Acids, Riboflavin and Thiamin in Late Summer Milk. Jour. Dairy Sci., 27: 849–855. 1944.

(27) HOLMES, A. D., KUZMESKI, J. W., LINDQUIST, H. G., AND RODMAN, H. B. Goat's Milk as a Source of Bone-building Minerals for Infant Feeding. Amer. Jour. Dis. Children, 71: 647–653. 1946.

(28) HOLMES, A. D., AND LINDQUIST, H. G. Some Characteristics of Mare's Colostrum and Milk. Amer. Dietet. Assoc. Jour. In press.

(29) HOLMES, A. D., LINDQUIST, H. G., JONES, C. P., AND WERTZ, ANNE W. Effect of High-Temperature-Short-Time Pasteurization on the Ascorbic Acid, Riboflavin and Thiamin Content of Milk. Jour. Dairy Sci., 28: 29–33. 1945.

(30) HOLMES, A. D., LINDQUIST, H. G., JONES, C. P., WERTZ, ANNE W., ESSELEN, KATHERINE, McKEY, BEULA V., AND FULLER, EVELYN. The Vitamin Content of Commercial Winter Goat's Milk. New England Jour. Med., 232: 72–76. 1945.

(31) HOLMES, ARTHUR D., McKEY, BEULA V., WERTZ, ANNE W., LINDQUIST, H. G., AND PARKINSON, L. R. The Vitamin Content of Mare's Milk. Jour. Dairy Sci., 29: 163–171. 1946.

(32) HOLMES, A. D., TRIPP, F., WOELFFER, E. A., AND SATTERFIELD, G. H. A Study of Breed and Seasonal Variations in the Ascorbic Acid Content of Certified Milk from Guernseys and Holsteins. Jour. Nutr., 17: 187–198. 1939.

(33) HOLMES, JULIA OUTHOUSE. The Requirement for Calcium during Growth. Nutr. Abs. and Rev., 14: 597–612. 1944–1945.

(34) HUNZIKER, O. F. Condensed Milk and Milk Powder. 6th Ed. Published by author, LaGrange, Ill. 1946.

(35) INGALLS, T. H., DRAPER, RUTH, AND TEEL, H. M. Vitamin C in Human Pregnancy and Lactation. II. Studies during Lactation. Amer. Jour. Dis. Children, 56: 1011–1019. 1938.

(36) KON, S. K. The Chemical Composition and Nutritive Value of Milk and Milk Products. Chem. and Indus., 62: 478–481. 1943.

(37) KONIG, J. Chemie der Nahrungs and Genussmittel. 5th Ed. Julius Springer. 1920. (Cited by Associates of Rogers.)

(38) KOTHAVALLA, Z. R., AND GILL, H. S. The Effect of Various Factors on the Vitamin C Content of the Milk of Cows and Buffaloes. Indian Jour. Vet. Sci. and Anim. Husb., 13: 35–43. 1943. (Chem. Abs., 39: 2340. 1945.)

(39) LINTON, R. G. The Composition of Mare's Milk. Jour. Agr. Sci., 21: 669. 1931.

(40) LINTON, R. G. The Composition of Mare's Milk. II. The Variation in Composition during Lactation. III. The Influence of Intra-mammary Pressure on the Composition of Milk with Special Reference to the Mare and Cow. Jour. Dairy Res., 8: 143–172. 1937.

(41) MACOLA, B. A., AND FAZIO, CARMELO. Ca and Mg of the Milk Sold in Cordoba, Argentina. Rev. Soc. Argentina Biol., 20: 649–658. 1944. (Chem. Abs., 39: 2345. 1945.)

(42) MASEK, J. Milk from the Wild Horse Przewalski. Chem. Obzor., 13: 137. 1938. (Chem. Abs., 33: 1379. 1939.)

(43) MAYNARD, L. A. Animal Nutrition. McGraw-Hill Book Co., Inc., New York. 1937.

(44) MORRISON, F. B. Feeds and Feeding. 20th Ed. The Morrison Publishing Co., Ithaca, N. Y. 1936.

(45) MUNKS, BERTHA, ROBINSON, A., WILLIAMS, H. H., AND MACY, ICIE G. Human Milk Studies. XXV. Ascorbic Acid and Dehydroascorbic Acid in Colostrum and Mature Human Milk. Amer. Jour. Dis. Children, 70: 176–181. 1945.

(46) NEHRING, K., AND WACHHOLDER, K. The Influence of Fodder on the Vitamin C Content of Cow Milk. Vitamine u. Hormone, 2: 400–416. 1942. (Chem. Abs., 38: 6419. 1944.)

(47) NIMS, BETTY, MACY, ICIE G., HUNSCHER, HELEN A., AND BROWN, MINERVA B. Human Milk Studies. X. Daily and Monthly Variations in Milk Components as Observed in Two Successive Lactation Periods. Amer. Jour. Dis. Children, 43: 1062–1076. 1932.

(48) NOTTBOHM, F. E. Milchw. Forsch., 4: 336. 1927. (Cited by Associates of Rogers.)

(49) ORLA-JENSEN, S. Ann. Agr. de la Suisse, 5–6, 125, 291. 1904–1905. (Cited by Associates of Rogers.)

(50) OVERMANN, O. R., SANMANN, F. P., AND WRIGHT, K. E. Studies of the Composition of Milk. Ill. Agr. Expt. Sta. Bul. 325. 1929.

(51) PAPP, G. The Milk of Mares. Allat. Lapok, 62: 6–9, 24–26, 44–46. 1939. (Chem. Abs., 33: 3435. 1939.)

(52) PLATON, B., AND SJOSTRUM, G. The Composition of Swedish Dairy Milk and of Milk from Swedish Goat-breeding Station. Madd. Statens Mejeriforsok, No. 4. 1940. (Chem. Abs., 39: 2344. 1945.)

(53) QUESADA, RAFAEL. The Vitamin C Content of Mother's Milk. Rev. asoc. bioquin Argentina, 5: 24–26. 1940. (Chem. Abs., 36: 140. 1942.)

(54) RASMUSSEN, R., BOGART, R., AND MAYNARD, L. A. Ascorbic Acid Content of Milk of Various Species as Influenced by Ascorbic Acid Injections and Diet. Soc. Expt. Biol. and Med. Proc., 39: 502–505. 1938.

(55) RICHMOND, MARTHA S., GRINNELLS, C. D., AND SATTERFIELD, G. H. Ascorbic Acid in Goats' Milk, Blood and Tissues. N. C. Agr. Expt. Sta. Tech. Bul. 68. 1942.

(56) RUDRA, M. N. Distribution of Vitamin C in Different Parts of Common Indian Foodstuffs. Biochem. Jour., 30: 701–703. 1936.

(57) SCHEPANG, W. Diss. Leipzig. 1917. (Cited by Associates of Rogers.)

(58) SCHRODT, M., AND HANSEN, H. Landw. Vers.-Sta., 31: 55. 1885. (Cited by Associates of Rogers.)

(59) SELLIG, IVA, AND KING, C. G. The Vitamin C Content of Human Milk and Its Variation with Diet. Jour. Nutr., 11: 599–606. 1936.

(60) SHERMAN, HENRY C. Chemistry of Food and Nutrition. 6th Ed. The Macmillan Co., N. Y. 1941.

(61) SHERMAN, HENRY C., AND LANFORD, CAROLINE SHERMAN. Essentials of Nutrition. The Macmillan Co., N. Y. 1943.

(62) SINKKO, E. I. The Relation of Infant Mortality and Mortinatality to the Seasonal Variations in Vitamin C Content of Breast Milk in Finland. Acta. Paedict., 21: 407–427. 1937. (Chem. Abs., 33: 692. 1939.)

(63) SOMMER, H. H., AND HART, E. B. The Heat Coagulation of Milk. Jour. Biol. Chem., 40: 137–151. 1919.

(64) SZABO, FERENC. Chemical Composition of Elephant Milk. Állat. Lapok, 67: 56–57. 1944. (Chem. Abs., 40: 7341. 1946.)

(65) TURNER, C. W., AND GARRISON, E. R. Vitamin C Content and Curd Tension of Goat's Milk. Mo. Agr. Expt. Sta. Bul. 413. 1940.

(66) VAN SLYKE, L. L. Modern Methods of Testing Milk and Milk Products. 3rd Ed. Orange Judd Publishing Co., N. Y. & London. 1927.

(67) VIETH, P. On the Composition of Mare's Milk and Koumiss. Analyst, 10: 218–221. 1885.

(68) WHITTLE, E. G. Goat's Milk and Human Milk. Analyst, 68: 247. 1943.

(69) WIDENBAUR, F., AND KÜHNER, A. Ascorbic Acid Studies on Lactating Women. Ztschr. f. Vitaminforsch., 6: 50–75. 1937. (Chem. Abs., 31: 2655. 1937.)

(70) WOLL, F. W. A Handbook for Farmers and Dairymen. John Wiley & Sons, Inc., N. Y. 1914.

(71) WRIGHT, P. A., DEYSHER, E. F., AND CARY, C. A. Variations in the Composition of Milk. Yearbook of Agriculture, U. S. Dept. Agr.: 639–648. 1939.

FLAVONES AND FLAVONE DERIVATIVES AS ANTIOXIDANTS

G. A. RICHARDSON, M. S. EL–RAFEY,[1] AND M. L. LONG

Division of Dairy Industry, College of Agriculture, Davis, California

The demands of war lent encouragement to the search for acceptable inhibitors of oxidative deterioration of food fats. This impetus resulted in the discovery of many effective processes and chemical agents, most of which have been patented but few of which have been acted upon by the Food and Drug officials.

The literature dealing with antioxidants has been covered by several reviews (6, 8, 9, 22, 26, 36, 37) and the characteristics and limitations of antioxidants and synergists have been discussed by Mattill (32), Golumbic (14), and others.[2] A partial list of antioxidants has been prepared (2).

The resistance of dry milk fat to oxidation has been shown to be due in part to its content of reducing substances (11) as determined by the Emmerie-Engel reagent. This reagent was found to be non-specific for a-tocopherol, a fact now universally recognized. It recently has been modified to determine the solubility in fats of several phenolic antioxidants (29). Experiments with this reagent suggested that reducing substances or components of oxidation-reduction systems that occur naturally in foods and that have food value in themselves should be ideal for incorporation into fats or fat-containing foods to serve as protectors against oxidation.

Reducing substances have been found among citrus juices, citrus peel, flower petals, rose hips and many other naturally occurring materials. Shrader and Johnson (44) recognized in orange juice distinct zones of oxidizing and reducing effects, the reducing effects being associated with the pigments. Svirbely and Szent-Györgyi (45) attributed part of the reducing value of orange juice to phenolic compounds later identified as flavonols. Hamburger and Joslyn (18) and Joslyn and Marsh (23), in studies related to browning of citrus juices, considered that among the reducing substances present in the juice were ascorbic acid and flavonols which might serve as a defense against browning. They believed that all of the reduced ascorbic acid first must be oxidized before the unknown reducing substance can itself be oxidized. This is in harmony with Golumbic's view (14). The flavones are known to oxidize ascorbic to dehydroascorbic acid (24).

Frankenthal (12), investigating the methylene blue reducing system of Palestine orange peels, found that the peel juice contained two factors not

adsorbed on animal charcoal and also an iodine-reducing, heat-stable factor which is adsorbed on animal charcoal and which is not ascorbic acid. The author recognized an oxidizing system in peel juice which acted upon ascorbic acid. Supporting evidence that flavone or flavone-like pigments contribute to the total reducing power of orange and raisin juices was contributed by Gatet (13), who studied the reducing power of quercetin, quercitrin, and their oxidation products. The impurity accompanying the flavones was thought to be a contributing factor. The reducing activity of these juices and of the flavone-type solutions, as measured by the 2-6,dichlorophenolindophenol method, was increased by first oxidizing in air at pH 8, followed by reduction by cysteine at pH 4. This may help to explain the beneficial results obtained by Shrader and Johnson (44) when oxygen was bubbled through certain batches of orange juice prior to packaging.

The belief that the flavone and flavanone pigments participate in oxidation-reduction reactions is strengthened by the work of Wawra and Webb (48) with citrin in which evidence was presented to show that citrin consists of hesperidin and the chalcone of hesperidin existing in equilibrium in an aqueous medium. In nature they probably exist as protein complexes and serve as an oxidation-reduction enzyme. The chalcone was shown to be capable of being reversibly oxidized and reduced. A small amount of quercitrin is believed to accompany the citrin, especially in the citrus fruit (41).

A review of the literature indicates that no attempt has been made to utilize the flavones or other phenyl-benzopyrone derivatives as antioxidants for animal fats. Greenbank and Holm (16) reported that quercetin seemed to possess antioxidant properties for cottonseed oil; Bradway and Mattill (5) found quercetin from quercitrin to be antioxygenic for a mixture of lard and cod-liver oil.

From the physiological, pharmacological, and nutritional aspects, the addition of flavones and their derivatives to foods appears acceptable and beneficial. They have been reported as vitamin P in a wide variety of foods (3, 38, 43, 47) following the postulation of Szent-Györgyi (46) that citrin, a mixture of flavones, possessed vitamin P activity. Many workers have used the biological technique to determine the vitamin P potency of fruits and vegetables (3, 41, 42); others have employed the chemical method of Lorenz and Arnold (28). Weatherby and Cheng (49) utilized the boric-citric acid reagent and reported the flavone or quercetin-like values of food products in terms of quercetin equivalents. Lemon peel ranked highest among the materials tested.

The occurrence, chemical nature, and vitamin P activity of several members of the flavone group have been reviewed (20, 27, 43). Direct evidence that the flavonol quercetin enhances the nutritive value of butter oil has been reported by El-Rafey et al. (10). This is in harmony with the growing appreciation of the role of antioxidants in fat, vitamin A, and carotene utilization.

Regardless of whether the flavone-type compounds complement the physiological activity of ascorbic acid, there seems to be accepted evidence that most of the representative compounds studied have beneficial pharmacological effects. The authors feel incompetent to analyze the extensive medical literature dealing with use of these compounds as therapeutic agents. They appear to have little or no toxicity, to be non-accumulative (17), and to have nutritional and medical significance (1, 31).

EXPERIMETAL

Substrates. Dry milk fat or butter oil was prepared by churning fresh pasteurized cream, melting the butter at 60° C., and decanting and filtering the resulting fat at 60° C. This fat was divided into small portions and refrigerated until required. The lard was prepared by melting at 60° C. fresh pure lard, obtained in the local market, filtering and refrigerating. For the experiments with milk, 2 p.p.m. of copper as copper sulfate were added to pasteurized winter milk, susceptible to copper-induced oxidation.

Antioxidants. These contained the phenyl-benzo-gamma-pyrone nucleus, usually with hydroxyl, glyco, or methoxy groups or the chalcone isomer. Quercetin, quercitrin, rutin, hesperidin, methylated hesperidin, hesperidin-chalcone, and others of the flavone group were studied.[3] Attempts to isolate and purify the individual components of Szent-Györgyi's citrin (46) were unsuccessful. Difficulty was experienced in separating and eluting the fractions in the Mager method (30). Recent methods should prove more fruitful (21, 39).

Incorporation of antioxidants. Most of the antioxidants studied were more soluble in alcohol than in water or fat and hence were dissolved in either hot ethanol or glycerol before being incorporated into either a small quantity of fat in a relatively high concentration or directly into the fat in the concentration desired. The alcohol then was evaporated off in partial vacuum at from 90 to 96° C. In the experiments with milk, 20 mg. of the antioxidants dissolved or suspended in 5 ml. of hot water were added to 200 ml. of milk. The excess of the material settled out during the storage period.

For the experiments in which quercetin was compared with antioxidants native to milk fat, stock solutions were prepared as follows:

A. *Soya bean phospholipid.* Commercial soya bean lecithin[4] was purified by dissolving twice in ether and precipitating with acetone. Eight grams, dissolved in 5 ml. of chloroform, were added to 400 ml. of milk fat to give a 2 per cent solution.

---

[3] Quercetin, Eastman Kodak Co. 1635; quercitrin, Eastman Kodak Co. T1629, purified (34); rutin, courtesy of Dr. J. F. Crouch, Eastern Regional Res. Lab., Philadelphia; hesperidin-chalcone, methylated chalcone, and hesperidin, courtesy of California Fruit Growers Exchange.

[4] Courtesy of Dr. Eichberg, American Lecithin Company, Elmhurst, New York.

B. *Tocopherol.* 0.1 g. α-tocopherol, dissolved in 1 ml. of chloroform was added to 100 ml. of milk fat to give a 0.1 per cent solution.

C. *Quercetin.* 0.1 g. quercetin, dissolved in 2 ml. hot absolute alcohol was added to 100 ml. of milk fat to give a 0.1 per cent solution.

The solvent was removed as described.

Incubation. Fat stabilities were determined by the open jar method, in which a uniform amount of the fat is placed in similar containers and incubated uncovered. The temperature conditions were secured by a controlled oil bath, electric oven, or refrigerated cabinet. The cabinet was maintained at approximately 10° C. for the milk samples.

Tests for stability. Peroxide values were determined by the Henderson and Young modification (19) of the Wheeler method (50). Carotene losses were measured as decreases in optical density (10% solution of the fat in gasoline, 1.6 cm. cell, 440 mμ wave length, 35 mμ band, 20° C.) using the Coleman, Model 11, spectrophotometer. The milk samples were scored for flavor as unknowns.

<div align="center">RESULTS</div>

Effect of concentration of antioxidant. Preliminary trials having shown that quercetin is antioxygenic for milk fat and for lard, experiments were made to determine an effective concentration for its use. Figures 1 and 2 show that a concentration of 3 mg. per 100 g., while affording protection, is not as adequate as a concentration of 15 or 30 mg. per 100 g. at an incubation temperature of 82° C. The figures also show that the incorporation of 15 mg. quercetin in 100 g. fat extended the times required to reach a peroxide value of 5 for milk fat or 10 for lard (respective arbitrarily chosen ends of the induction periods) from 30 to over 144 hours for milk fat and from 26 hours to over 96 hours for lard. Competent judges were unable to detect by taste or color the presence of at least 30 mg. per cent of quercetin in butter oil, lard, or butter-like preparations made from them.

Protection of milk fat in storage. It was shown previously (11) that soya bean phospholipids and α-tocopherol, either alone or in synergistical combination were antioxygenic for butter oil held at 79.5° C. In order to determine the effectiveness of these compounds at a lower temperature and to compare their effectiveness with that of quercetin, a milk fat was isolated in the manner described except that melting and filtration were carried out at 41–47° C. rather than at 60° C.

Using the stock solutions A, B, or C, or combinations of A and B, samples of this fat were prepared to contain from 0.01 to 0.1 per cent added phospholipid (mostly lecithin), from 0.003 to 0.015 per cent added α-tocopherol, or from 0.003 to 0.015 per cent quercetin. Synergistic combinations were prepared containing 0.01 per cent phospholipid and 0.003 per cent α-tocopherol; 0.1 per cent phospholipids and 0.003 per cent α-tocopherol; or 0.1 per cent

phospholipid and 0.015 per cent α-tocopherol, in addition to those naturally present.

With the exception of samples 1 and 4 which were stored in test tubes
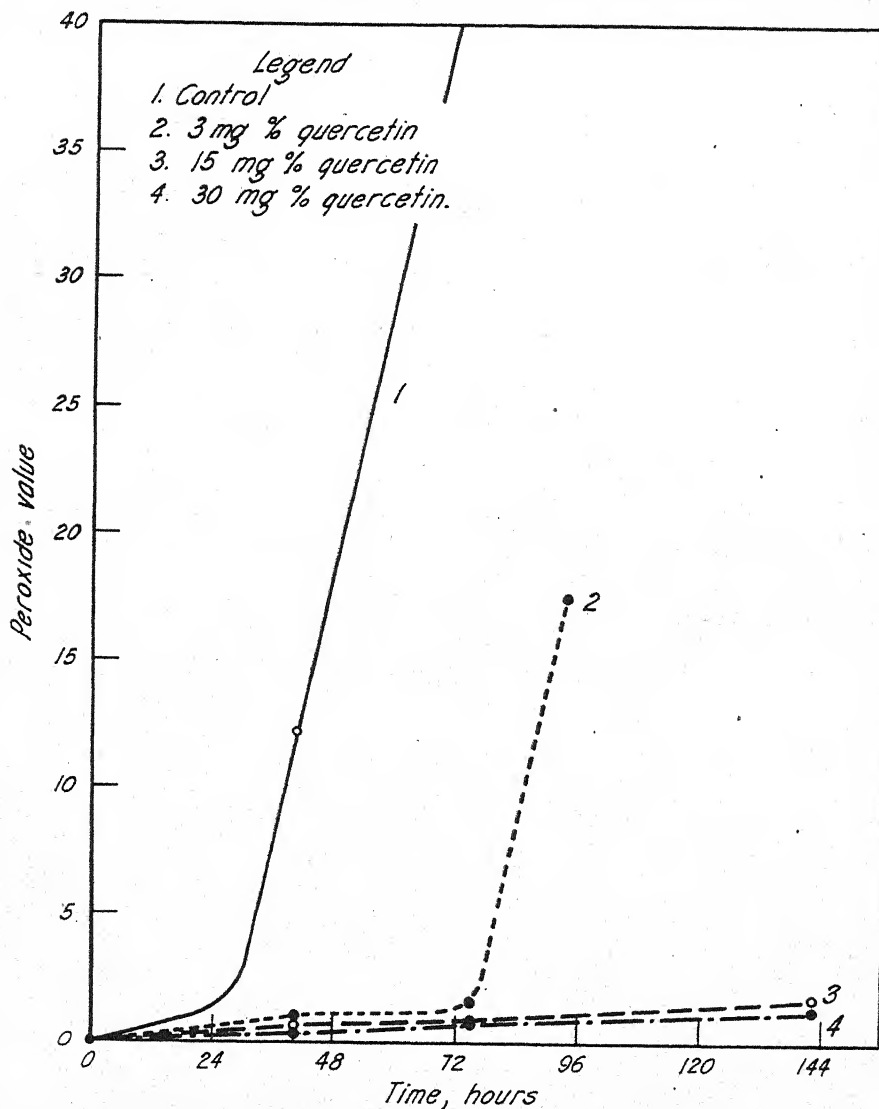
FIG. 1. The effect of adding quercetin to butter oil on its resistance to oxidation at 82° C.

at 4.5–10° C. (40–50° F.), approximately 100 ml. aliquots of each sample, contained in one-quarter pint milk bottles, with loosely-fitting caps, were stored at 40–50° F. from July 27, 1943, until about October 1, 1943.   They

then were placed in a wooden cabinet at room temperature. At intervals the fats were melted and samples were taken for peroxide and color determinations. The results are shown in table 1. The experiment does not lend
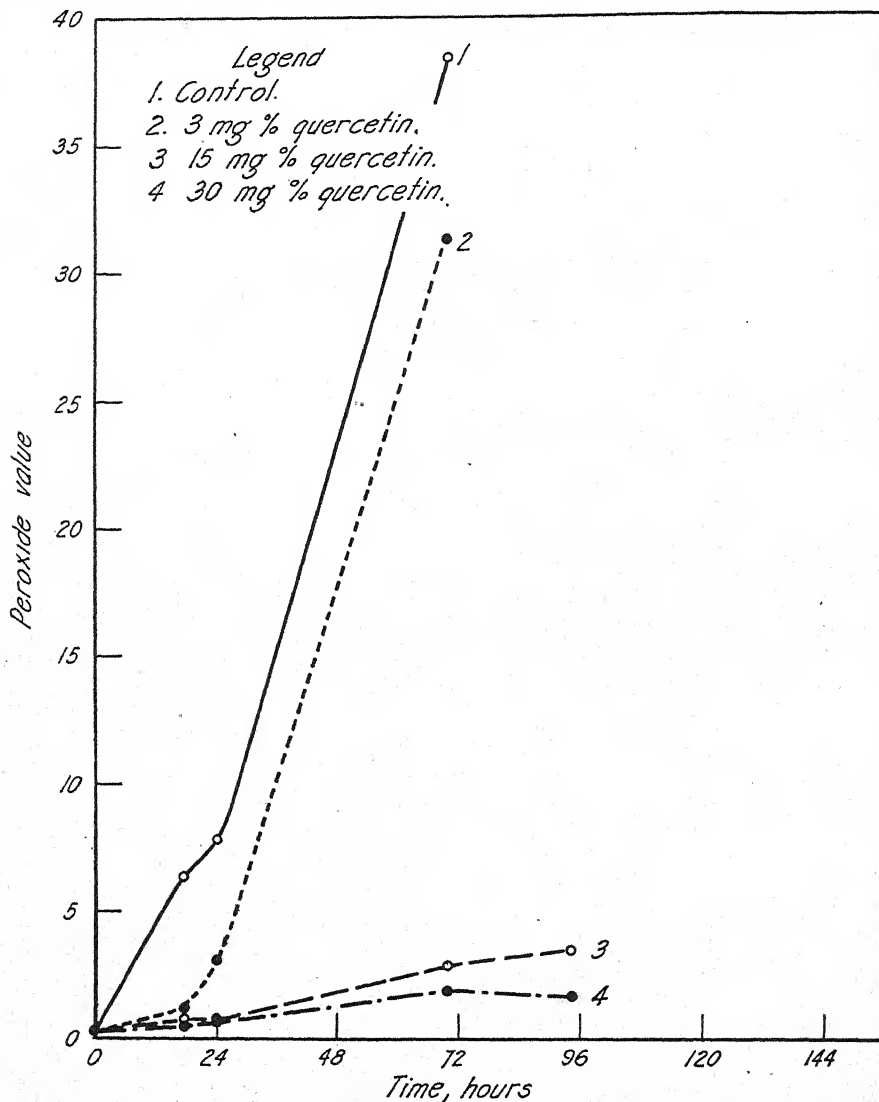
FIG. 2. The effect of adding quercetin to lard on its resistance to oxidation at 82° C.

itself to an evaluation of absolute protection factors of the antioxidants, but the data suggest that α-tocopherol added to milk fat, normally containing tocopherol(2–3 mg./100 g.), has no protective value under these conditions

TABLE 1

Effect of antioxidants on the storage life of milk fat

(Samples were prepared in July, 1943, held cold until Oct, 1943, and subsequently stored at room temperatures)

Sample no.	Description	Peroxide no.* and optical densities† (in parentheses)					
		Feb. 5, 1944	June 2, 1944	Sept. 26, 1944	May 7, 1945	Sept. 8, 1945	July 22, 1946
1	Control, milk fat held at 40–50° F.	....	0.75 (0.255)	2.5	70 (white)	....	....
2	Milk fat plus 0.01% P‡	0.96	1.91 (0.243)	3.45 (0.21)	(white) (0.075)	....	....
3	Milk fat plus 0.05% P	0.63	0.73 (0.26)	2.35 (0.24)	18.4 (0.05)	....	....
4	Milk fat plus 0.1% P held at 40–50° F.	....	0.0 (0.26)	0.0 (0.26)	1.52	....	....
5	Milk fat plus 3 mg.% T‡	1.1	3.71 (0.20)	8.16 (0.15)	(white)	....	....
6	Milk fat plus 9 mg.% T	1.7	6.39 (0.23)	12.15 (0.13)	(white-0.08)	....	....
7	Milk fat plus 15 mg.% T	2.94	14.5 (0.255)	16.26 (0.115)	(white)	....	....
8	Milk fat plus 0.01% P and 3 mg.% T	0.37	1.3 (0.27)	3.56 (0.22)	(white-0.075)	....	....
9	Milk fat plus 0.1% P and 3 mg.% T	0.24	0.92 (0.264)	2.83 (0.23)	Almost white	....	....
10	Milk fat plus 0.1% P and 15 mg.% T	0.48	1.98 (0.256)	7.1 (0.23)	(0.07)	....	....
11	Milk fat plus 3 mg.% Q‡	0.55	0.83 (0.275)	1.33 (0.25)	3.88 (0.205)	6.43	....
12	Milk fat plus 15 mg.% Q	0.24	0.53 (0.31)	0.89 (0.29)	1.96 (0.30)	2.46	3.18
A	Milk fat plus 2% P	....	....	0.0 (0.27)	0.84 (0.25)	2.02	3.83
B	Milk fat plus 0.1% T	....	....	35.1 (0.21)	(0.06)	....	....
C	Milk fat plus 0.1% Q	....	....	0.0 (0.29)	0.82 (0.265)	1.44	1.35

* Milliequivalents per kg. of fat.
† Optical density (10% solution of fat in gasoline: 1.6 cm. cell, 440 mμ wave length, 35 mμ band, 20° C.)
‡ P—phospholipid; T—α-tocopherol; Q—quercetin.

of storage. It even appears prooxygenic. Soya bean phospholipid (mainly lecithin), of itself and in combination with a-tocopherol, is definitely antioxygenic for milk fat. The proportion of 0.003 per cent tocopherol to 0.1 per cent phospholipid (sample 9) appears a desirable addition.

The phospholipid content of the control fat was 0.0214 per cent (calculated as lecithin). Assuming that milk fat contains 0.0025 per cent tocopherol, sample 9 had a tocopherol: phospholipid ratio of approximately 1 : 22, whereas the ratio for the control fat was approximately 1 : 8.6.

The effectiveness of quercetin, especially in the 0.015 per cent concentration, is outstanding. The 0.1 per cent concentration, while effective, is in considerable excess of the solubility of quercetin in fat. The protective action of quercetin for carotene will be the subject of a later paper.

The relative effectiveness of quercetin and commercial quercitrin. Quercetin and quercitrin (unpurified), Eastman Kodak Co., were added to milk fat and to filtered lard colored with carotene by the addition of 0.4 ml. of a concentrate[5] to 200 ml. lard. After removal of the solvent, ethanol, 50 ml. samples, in 200-ml. straight-walled bottles, were incubated in the dark at 47–50° C. Table 2 shows that quercetin is more effective than quercitrin on the weight basis. The latter is a quercetin 3-rhamnoside and has a quercetin equivalent of 354 mg. per gram as determined by the method of Weatherby and Cheng (49). On this basis, then, the quercitrin was present only in a quercetin-equivalent concentration of approximately 7 and 10.5 mg. per cent in the milk fat and lard, respectively.

Other flavone-type compounds as antioxidants. Following the procedure suggested by Wawra and Webb (48), crude preparations of hesperidin and its chalcone were made from air-dried orange and lemon peels. Their identity and purity were not established. The compounds, dissolved in ethanol, were incorporated by the described procedure, but the samples were subjected to a fluctuating temperature of 72–75° C. for the first 72 hours, except for about 6 hours at 90° C. They then were incubated at 48–50° C. The results in table 3 show that rutin from tobacco (quercetin-equivalent approximately 300 mg. per gram) has lower ·antioxygenic properties than quercitrin. Sample 4, containing rutin and a crude mixture of hesperidin and its chalcone, had a pronounced fruity odor after the first heating. This largely disappeared as incubation progressed. The sample showed remarkable resistance to the accumulation of peroxides. Sample 6 indicates the potential antioxygenic properties in the chalcone. This is demonstrated more conclusively in table 4. Partially purified flavanones (hesperidin, hesperidin-chalcone, methylated hesperidin-chalcone) and a lemon peel infusion concentrate prepared by the method of Szent-Györgyi for vitamin P studies were kindly furnished by California Fruit Growers Exchange. These were incorporated into milk fat in the manner already described, and

[5] Courtesy of H. M. Barnett, Barnett Labs., Long Beach, California.

TABLE 2

Effect of quercetin and quercitrin on the keeping quality at 47–50° C. of milk fat and of lard containing carotene

(Approximately 9,000 I.U. vitamin A per lb.)

Sample no.	Description	Peroxide values and optical densities* (in parentheses)									
		Dec. 17, 1943	Dec. 23, 1943	Dec. 27, 1943	Dec. 31, 1943	Jan. 11, 1944	Feb. 2, 1944	Mar. 2, 1944	Mar. 25, 1944	Mar. 28, 1944	Apr. 7, 1944
B	Milk fat untreated	(0.30)	0.26	......	......	......	......	......	......	......	......
L	Lard plus carotene untreated	(0.41)	0.65	......	......	......	......	......	......	......	......
1‡	B-control	(0.28)	0.48 (0.28)	3.7 (0.22)	22.8 (0.06)	110.7 (0.02)	217 (0.02)	......	......	......	......
3	B plus 20 mg.% QT†	(0.30)	0.86 (0.32)	1.94 (0.34)	1.91 (0.29)	2.61 (0.27)	35.4 (0)	......	......	......	......
4	B plus 20 mg.% Q	(0.31)	0.36 (0.33)	...... (0.35)	1.03 (0.30)	1.9 (0.33)	3.44 (0.16)	3.72 (0.11)	6.47 (0.1)	25.4 (0.05)	113 (0.01)
5	L-control	(0.39)	1.04 (0.38)	14.9 (0.09)	43.3 (0.03)	97.4 (0.09)	150 (0.02)	......	......	......	......
7	L plus 30 mg.% Q	(0.50)	0.88 (0.52)	...... (0.40)	1.41 (0.47)	2.61	3.54 (0.31)	4.98 (0.21)	7.68 (0.19)	9.49 (0.11)	15.3 (0.08)
8	L plus 30 mg.% QT	(0.50)	2.02 (0.51)	2.07 (0.50)	3.67 (0.43)	25.36 (0.16)	136 (0.04)	......	......	......	......

* Optical density (10 per cent solution of fat in gasoline; 1.6 cm. cell, 440 mμ wave length, 35 mμ band, 20° C.).
† Q—Quercetin; QT—quercitrin (not purified).
‡ Samples 1 and 5 received the same pre-incubation treatment as samples 3, 4, 7, and 8.

TABLE 3

Effect of quercitrin, rutin, and hesperidin chalcone on the keeping quality of milk fat at 40–50° C.
(1944)

Sample no.	Description*	Peroxide values (me/kg. fat)										
		May 12	May 15	May 17	May 23	May 26	June 9	June 15	June 23	June 30	July 24	Oct. 4
1	QT 30 mg.%	1.37	1.58	1.60	1.93	2.11	2.66	3.0	3.41	3.49	6.72	......
2	R 30 mg.%	1.71	2.35	2.46	3.4	12.94	......	......	......	......	......	......
4	R 30 mg.% plus HC	0.0	0.0	0.0	0.0	0.0	......	......	......	......	......	3.36
5	R 20 mg.% (dry)	1.87	2.26	2.64	15.4	42.2	......	......	......	......	0.8	......
6	Control	2.03	9.39	23.5	......	......	18.5	......	......	......	......	......
7	HC conc. not determined	1.33	1.93	1.93	2.29	2.52	......	......	......	......	......	......

* QT—quercitrin from lemon flavine (34); R—rutin from tobacco; HC—hesperidin chalcone, a purified extract of orange and lemon peels.

TABLE 4

Hesperidin and hesperidin chalcone as antioxidants for milk fat
(1945)

		Peroxide values (me/kg. fat)									
	Initial Oct. 2 3 p.m.	Oct. 3		Oct. 4		Oct. 5		Oct. 6 11 a.m.	Oct. 10	Oct. 12	Oct. 16
		1:20 p.m.	4:15 p.m.	9 a.m.	3:45 p.m.	11 a.m.	4 p.m.				
Experiment 1											
Control	1.7	2.32	2.41	7.04	......	......	......	......	......	......	......
HC, 10 mg./30 g. fat	......	......	......	4.81	9.8	11.95	......	......	......	......	......
HC, 20 mg./30 g. fat	......	......	......	2.62	2.48	3.98	6.24	13.75	......	......	......
Quercetin 10 mg./30 g. fat	......	......	......	2.08	1.96	2.11	1.91	2.82	2.87	3.52	4.88

	Initial Oct. 4 3 p.m.	Oct. 5		Oct. 6 11 a.m.	Oct. 8 4 p.m.	Oct. 9 4 p.m.	Oct. 10 4 p.m.
		10 a.m.	3:45 p.m.				
Experiment 2							
Control	1.31	2.5	3.96	6.9	8.32	13.25	......
Crude H ca. 50 mg./30 g. fat	......	2.45	2.29	4.54	7.58	9.54	13.15
Lemon peel infusion conc. ca. 40 mg./30 g. fat	......	2.23	2.41	2.73	2.76	6.35	10.11

HC—Hesperidin chalcone—Courtesy California Fruit Growers Exchange.
H—Hesperidin, crude—Courtesy California Fruit Growers Exchange.
Lemon peel infusion for vitamin P studies—Courtesy California Fruit Growers Exchange.

the fat samples were incubated at from 60 to 80° C. during the day and chilled during the night or over the weekend. The results indicate that hesperidin has little or no antioxidant value. Other experiments, not reported, showed that the methylated chalcone probably is too stable to serve as an antioxidant. The data, however, show that the natural chalcone protects milk fat against oxidation.

Inhibiting oxidation in susceptible milk. Twenty mg. of the compounds listed in table 5 were added to 200 ml. of warm pasteurized susceptible milk

TABLE 5

Flavone-type compounds as antioxidants for milk susceptible to copper-induced oxidation

Sample no.	Antioxidant	Numerical score*	Comments
1	Key, no copper	21	Old, very sl. oxidized
2	Hesperidin	18	Oxidized
3	Hesperidin-chalcone-protein complex†	21	Sl. foreign
4	Rutin	21	Old
5	Quercitrin	22	Lacks freshness
6	Control, with copper	18	Oxidized
7	Lemon peel infusion concentrate (vitamin P)	20	Old, sl. oxidized
8	Quercetin	22	Old, lacks freshness
9	Same as 7, except one-half concentration	19	Oxidized
11	Hesperidin chalcone	20	Foreign flavor

* Flavor and odor; 25, no criticism.
† Prepared by method of Wawra and Webb (48).

along with 2 p.p.m. of copper. A control was prepared omitting the compound; a key sample, containing no added copper, was included. All were cooled and placed in a refrigerated cabinet (10° C.) and scored as unknowns on the third day. The scores and comments of one of the judges, concurred in by the other judges, are shown in the table. All of the flavones and flavanones studied inhibited the development of the oxidized flavor common to winter milk. Experiments are contemplated in which the antioxidant will be incorporated prior to pasteurization.

DISCUSSION

The flavones and flavone derivatives are distributed widely in the plant kingdom and occur as glycosides, in the free state, and in association with proteins and tannins. They all have the basic phenyl-benzo-gamma-pyrone structure, the pyrone nucleus apparently being responsible for their characteristic chemical activity. The key structures, according to Mayer and Cook (33), are as follows:
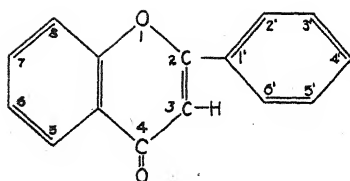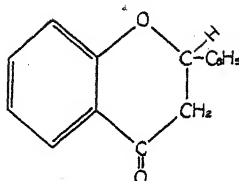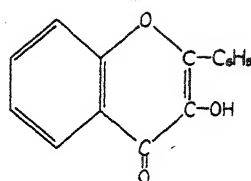
Flavone   Flavanone   Flavonol
                                     (enol form)
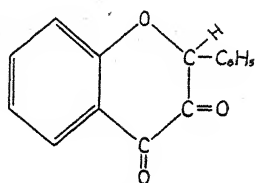
Flavonol   Chalcone
(keto form)
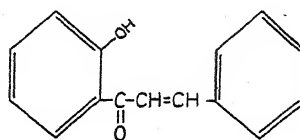
Mayer and Cook state that a flavanone may be dehydrogenated to a flavone and a chalcone may be converted to the flavonol by treatment with hydrogen peroxide. The chalcone in acid medium is believed to isomerize into the flavanone, the reverse taking place in the alkaline medium (48).

Of the flavone-type compounds studied, those giving a positive boro-citric test (51) were most efficient as antioxidants. This test apparently involves the

$$-\underset{\underset{O}{\parallel}}{C}-\underset{|}{C}=C-$$

group. Quercetin (3,5,7,3',4', pentahydroxy-flavone), quercitrin (3-rhamnoside of quercetin), rutin (3-rutinoside), and possibly the chalcone of hesperidin (48) all contain this grouping and all have antioxidant activity. It would seem logical, therefore, to infer that the labile pyrone confers antioxidant properties. The closed, saturated ring of hesperidin apparently does not satisfy this condition and it has very little, if any, antioxidant value, at least in the absence of a synergist of lower oxidation potential.

A theoretical discussion of the manner in which flavones and their derivatives protect a fat against oxidation is somewhat premature. Milk fat as usually isolated is afforded some protection by the phosphatides, tocopherols, and other reducing substances which it contains. These are difficult to remove without effecting changes in the fat itself. Carotene cannot be ignored. In milk itself the natural oxidation-reduction system or systems is not a simple one, even if ascorbic acid is excluded. Furthermore, individual flavones and flavanones are not easily isolated from their isomers and other naturally-occurring pigments.

The data are interpreted as supporting the modern concept of antioxidants. Very little seems to be known of the oxidation-reduction potentials of the flavones in a fat medium. The potential of a fat peroxide is not known, but that of a fresh fat may be about 1.0 volt (15). Gatet (13), by oxidizing quercetin in air at pH 8, followed by reduction with cysteine at pH 4, increased its apparent concentration toward 2,6-dichlorophenol-indophenol about four-fold. It would seem that the potential of this activated quercetin in a water-alcohol medium at pH 7 is somewhat less than 0.22 volt. If this is true, sulfhydryl compounds and possibly ascorbic acid might well be expected to act synergistically with these flavones. Sample 4, table 3, suggests that a combination of a flavone and a chalcone may be very effective in inhibiting the accumulation of peroxides. This aspect is being studied.

It is not known if ingested flavones or flavanones will be deposited in the body fat, carried in the blood stream, or secreted in the milk of animals, as appears to occur under certain conditions with tocopherol (7). None was found in the livers of rabbits (51) or in milk (35), and Robeznicks (40) was unable to establish either the presence or absence of flavones in the liver, kidney, or milk of animals. Feeding citrus molasses or dried citrus pulp to dairy cows has proved satisfactory and no ill effects on the milk flavor have been found (4, 25). If ingested flavones are found to be deposited in the body fat and secreted in milk, the authors are of the opinion that these ingested compounds would result in enhanced stability of meat products and improved keeping quality of winter milk and of whole milk products.

The lack of specific tests for flavones and flavanones, especially in the presence of interfering substances, coupled with the difficulty of securing foodstuffs free from flavones, makes conclusive results hard to obtain.

CONCLUSIONS

1. Flavones, as illustrated by quercetin, quercitrin, and rutin, have been shown to be effective antioxidants for milk fat and lard.

2. The flavanone glycoside hesperidin appears to have little or no antioxidant values; its chalcone is active.

3. It is suggested that the $-\underset{\underset{O}{\|}}{C}-\underset{}{C}=\underset{}{C}-$ group in the pyrone ring or in the open chalcone is responsible for the antioxidant activity.

REFERENCES

(1) ANONYMOUS. Citrin and Related Products. Nutr. Rev., 1: 214–216. 1943.

(2) ANONYMOUS. List of Antioxidants Proposed for the Preservation of Edible Fats (with a Selected Bibliography of Papers and Patents). Pub. A.I.C. -54, Bur. Agr. Chem., So. Reg. Res. Lab., New Orleans 19. 1944.

(3) BACHARACH, A. I., AND COATS, M. E. The Vitamin-P Activities of Citrus Fruits, Rose-Hips, Black Currants, and Some Fruit Products and Concentrates. Soc. Chem. Indus. Jour., Trans. and Commun., 63: 198–200. 1944.

(4) BECKER, R. B., ARNOLD, P. T. DIX, DAVIES, G. K., AND FOUTS, E. L. Citrus Molasses—a New Feed. Jour. Dairy Sci., 27: 269–273. 1944. (Also Chem. Abs., 36: 3282, 1942; 29: 4096, 1935; 35: 1464, 1941.)

(5) BRADWAY, E. M., AND MATTILL, H. A. The Association of Fat-soluble Vitamins and Antioxidants in Some Plant Tissues. Amer. Chem. Soc. Jour., 56: 2405–2408. 1934.

(6) BROWN, W. C., AND THURSTON, L. M. A Review of Oxidation in Milk and Milk Products as Related to Flavor. Jour. Dairy Sci., 23: 629–685. 1940.

(7) BURR, G. O., LUNDBERG, W. O., AND CHIPAULT, J. R. The Role of Various Substances in Stabilizing Animal Tissues. Oil and Soap, 23: 382–384. 1946.

(8) COMMITTEE ON FOOD RESEARCH—Conference on Deterioration of Fats and Oils (War Dept., U. S.) Army Service Forces, Q.M. Corps. Q.M.C. 17–7, pp. 47–153. 1945.

(9) DAUBERT, B. F. Bibliography and Review of Fat and Oil Deterioration. Quartermaster Food and Container Inst. Chicago. 1946.

(10) EL-RAFEY, M. S., HART, E. B., AND SOMMER, H. H. Wis. Agr. Expt. Sta. Bul. 465, p. 33. 1944.

(11) EL-RAFEY, M. S., RICHARDSON, G. A., AND HENDERSON, J. L. The Effect of the Method of Manufacture of Butteroil, on Its Keeping Quality. Jour. Dairy Sci., 27: 807–820. 1944.

(12) FRANKENTHAL, L. The Methylene Blue Reducing System of Palestine Orange Peels Investigated by the Thumberg Method. Enzymologia, 6: 287–306. 1939.

(13) GATET, M. L. Étude du Pouvoir Réducteur des Jus de Fruits au Cours de la Maturation. Enzymologia, 6: 375–386. 1939.

(14) GOLUMBIC, C. Kinetics of Antioxygenesis. Oil and Soap, 23: 184–186. 1946.

(15) GREENBANK, G. R. Committee on Food Research—Conference on Deterioration of Fats and Oils (War Dept., U. S.) Army Service Forces, Q.M. Corps. Q.M.C. 17–7, p. 97. 1945.

(16) GREENBANK, G. R., AND HOLM, G. E. Antioxidants for Fats and Oils. Indus. and Engin. Chem., 26: 243–245. 1934.

(17) GRIFFITH, J. Q., JR., COUCH, J. F., AND LINDAUER, M. A. Effect of Rutin on Increased Capillary Fragility in Man. Soc. Expt. Biol. and Med. Proc., 55: 228–229. 1944. (Also Abs. of Papers, 109th Meeting, Amer. Chem. Soc., pp. 60–61K. Apr., 1946; Pub. A,I.C.–115, East. Reg. Lab., U. S. Dept. Agr., Philadelphia. 1946.)

(18) HAMBURGER, J. J., AND JOSLYN, M. A. Auto-oxidation of Filtered Citrus Juices. Food Res., 6: 599–619. 1941.

(19) HENDERSON, J. L., AND YOUNG, H. A. Some Aspects of the Rate of Reaction of Oleic Acid with Oxygen. Jour. Phys. Chem., 46: 670–684. 1942.

(20) HIGBY, R. H. The Chemical Nature of Vitamin P. Amer. Pharm. Assoc. Jour., 32: 74–77. 1943.

(21) HIGBY, R. H. Hesperidin, U. S. Pat. 2,400,693 (to Calif. Fruit Growers Exch.), May 21, 1946.

(22) HILDITCH, T. P. Some Factors Affecting the Control of Oxidative Rancidity. Chem. and Indus., 8: 67–71. Feb., 1944.

(23) JOSLYN, M. A., AND MARSH, G. L. Browning of Orange Juice. Indus. and Engin. Chem., 27: 186–189. 1935.

(24) KING, C. G. Reactions of Ascorbic Acid in Vivo. Sympos. of Quant. Biol., 7: 137–147. 1939.

(25) LAVIGNE, N. F. Results of Recent Feeding Research Favor Dried Citrus Pulp for Dairy Cows. Milk Plant Monthly, 29(9): 69–71. 1940.

(26) LEA, C. H. Experiments in the Use of Antioxidants in Dry Edible Fat. Soc. Chem. Indus. Jour., 63: 107–112. 1944.

(27) LINDHEIMER, G. T., HINMAN, W. F., AND HALLIDAY, E. G. The Function and Occurrence of Citrin ("Vitamin P"). Amer. Dietet. Assoc. Jour., 18: 503–507. 1942.

(28) LORENZ, A. J., AND ARNOLD, L. J. Preparation and Estimation of Crude Citrin (Vitamin P) from Lemons. Food Res., 6: 151–156. 1941.

(29) LUNDBERG, W. O., AND HALVORSON, H. O. Observations on Solubilities and Other Properties of Antioxidants in Fats. Inst. of Food Technologists Proc., pp. 115–126. 1945.

(30) MAGER, A. Composition of Eriodictyol Glucosides. Ztschr. f. Physiol. Chem., 274: 109–115. 1942.

(31) MAJOVSKI, G. J., LESSER, A. J., LAWSON, H. C., CARNE, H. O., AND THIENES, C. H. Vascular Fragility and Permeability as Influenced by Various Agents. I. Jour. Pharmacol. and Expt. Ther., 80: 1–7. 1944.

(32) MATTILL, H. A. Anti-oxidants and Synergists. Oil and Soap, 22: 1–3. 1945.

(33) MAYER, F., AND COOK, A. H. The Chemistry of the Natural Coloring Matters. Reinhold Pub. Corp., N. Y., p. 162. 1943.

(34) MORROW, C. A., AND SANDSTROM, W. M. Biochemical Laboratory Methods. John Wiley and Sons, N. Y., pp. 231–232. 1935.

(35) NEUWEILER, W. Does Citrin (Vitamin P) Occur in Milk? Ztschr. f. Vitaminforsch, 9: 338–342. 1939. (Chem. Abs., 34: 2891. 1940.)

(36) OLCOTT, H. S., AND MATTILL, H. A. Constituents of Fats and Oils Affecting the Development of Rancidity. Chem. Rev., 29: 257–268. 1941.

(37) PISKUR, M. M. Review of Literature on Fats, Oils and Soap. Part II. Oil and Soap, 23: 12–27. 1946.

(38) POLLARD, A. The Vitamin P Activity of Black Currants. The Food Prods. Jour. and Amer. Food Mfgr., 24: 139–142. 1945.

(39) PRITCHETT, D. E., AND MERCHANT, H. E. The Purification of Hesperidin with Formamide. 1946. (Unpublished.)

(40) ROBEZNICKS, IRINA. Critical Observations on the Vitamin P Question. Ztschr. f. Vitaminforsch, 8: 27–31. 1938.

(41) RUSZNYÁK, ST., AND BENKÓ, S. The Vitamin Nature of the Flavones. Klin. Wchschr., 20: 1265–1267. 1941. (Also Science, 94: 25. 1941.)

(42) RUSZNYÁK, ST., AND SZENT-GYÖRGYI, A. Vitamin P. Flavonols as Vitamins. Nature, 138: 27. 1938.

(43) SCARBOROUGH, H. Observations on the Nature of Vitamin P Potency of Certain Foodstuffs. Biochem. Jour., 39: 271–278. 1945.

(44) SHRADER, J. H., AND JOHNSON, A. H. Freezing Orange Juice. Indus. and Engin. Chem., 26: 869–874. 1934.

(45) SVIRBELY, J. L., AND SZENT-GYÖRGYI, A. The Chemical Nature of Vitamin C. Biochem. Jour., 26: 865–870. 1932.

(46) SZENT-GYÖRGYI, A. Methoden zur Herstellung von Citrin. Hoppe-Seylers Ztschr. f. Physiol. Chem., 255: 126–131. 1938.

(47) Teng-Yi, Lo, and Chen, Shan-Ming. Vitamin P Content of Vegetables as Influenced by Chemical Treatment. Food Res., 11: 159–162. 1946. (Also: Chem. Abs., 35: 6007. 1941; 38: 176. 1944; 38: 3320. 1944.)

(48) Wawra, C. Z., and Webb, J. L. The Isolation of a New Oxidation-Reduction Enzyme from Lemon Peel (Vitamin P). Science, 96: 302–303. 1942.

(49) Weatherby, L. S., and Cheng, A. L. S. The Determination of Flavones or Quercetin-like Substances in Certain Naturally Occurring Products. Jour. Biol. Chem., 148: 707–709. 1943.

(50) Wheeler, D. H. Peroxide Formation as a Measure of Autoxidative Deterioration. Oil and Soap, 9: 89–97. 1932.

(51) Wilson, C. W., Weatherby, L. S., and Bock, W. Z. Determination of Certain Quercetin-like Substances. Indus. and Engin. Chem., Anal. Ed., 14: 425–426. 1942.

ABSTRACTS OF LITERATURE

BOOK REVIEW

1. **Modern Development of Chemotherapy.** E. HAVINGA, H. W. JULIUS, H. VELDSTRA, AND K. C. WINKLER. Edited by I. R. HOUWINK AND J. A. A. KETELAAR. 175 pages. $3.50. Elsevier Publishing Company, Inc., New York. 1946.

This publication represents one of a series of monographs on the progress of research in Holland during the war. These monographs, begun during the war, contain information most of which was kept secret until the end of the war.

The discussions include: (1) An introduction which emphasizes the important aspects of chemotherapy; (2) Bacteriological and physiochemical investigations on the mechanism of the action of the sulfonamides and of para-aminobenzoic acid; (3) Chemical investigations including synthesis and activity of sulfonamide derivatives and related compounds, action of sulfonamides on enzymes and analytical experiments on quantitative determination of sulfonamide derivatives and para-aminobenzoic acid; (4) Investigations on pharmacology, immunology, and clinico-therapeutical results including studies on streptococci, pneumococci, meningococci, gonococci, and other organisms; (5) Mycotherapy investigations on expansine, a product stemming from studies on antagonistic effect of antibiotics on plant pathogens.

The monographs are well written and presentations of data enhanced by numerous tables, figures, and structural formulas. P.R.E.

BACTERIOLOGY

2. **The Selective Action of Penicillin in the Isolation of *Brucella abortus* from Milk.** HELEN A. LACY, L. J. RODE, AND V. T. SCHUHARDT, Brucellosis Research Project of the Clayton Foundation, University of Texas, Austin, Texas. Jour. Bact., *52*, 3: 401. September, 1946. Abs. Proc. of Local Branches.

"Pooled milk samples from the four quadrants of the udder were collected in sterile test tubes by hand milking from 564 cows. The cream from these samples was plated in duplicate on the usual gentian violet tryptose agar (GVTA) and on this medium containing 1 Oxford unit of penicillin per cubic centimeter (PGVTA). *Brucella abortus* colonies were isolated a total of 87 times from the two media. In 58 instances *B. abortus* was isolated on the PGVTA and not on the duplicate GVTA. In only 1 instance was the reverse of this situation true. In 20 instances the PGVTA plates

A1

showed more than 50 *B. abortus* colonies, whereas not one of the GVTA plates showed more than 50 colonies. In 12 instances the PGVTA plate showed more than 50 colonies of *B. abortus,* whereas not a single colony was found on the duplicate GVTA plate. *B. abortus* was isolated from 86 (15.24 per cent) of the 564 samples on PGVTA and from 29 (5.14 per cent) of the samples on GVTA." D.P.G.

BUTTER

3. **Testing Sour Cream for Extraneous Matter.** KENNETH M. RENNER, Texas Technological College, Lubbock, Texas. Southern Dairy Products Jour., *38,* 4: 64. October, 1945.

The following methods of filtering four-ounce samples of sour cream were used: (1) Soda Solution (Illinois Method), (2) Dry Soda Method, (3) Acid (HCl) Method recommended by Prof. M. G. Pederson of Texas Technological College, (4) Texas Tech. Method (Calgon–Diversey–USN–Citric Acid), (5) Special Solution designated by the Diversey Company as X–300, (6) Special Powder Solution, designated by the Diversey Company as D–229.

The first four tests have been recently described by the American Butter Institute. Method 5 consists in adding nine ml. of Solution X–300 to the cream sample, stirring for one minute, thoroughly stirring in eight ounces of water at 180–190° F. and filtering. For Method 6 make a 10% solution of D–229 in distilled water, heat to 160–180° F., stir frequently, allow to cool, and filter. Add two ounces of this stock solution to a sample, mix, allow to stand two or three minutes, add eight ounces of 180–190° water, mix well, and filter.

All methods filtered ordinary farm cream successfully except that Method 1 did not filter 35–50% cream five days old and Method 2 was not successful at five days for 50% cream and at seven days for 35–40% cream. When one ounce of a 50% solution of citric acid was added to each gallon of water used for diluting the cream, Methods 1 and 2 were successful on Grade A cream until it was at least seven days old. The use of untreated water with a hardness exceeding 15 grains caused difficulty in the alkaline methods.

Good results were obtained from Method 1 when the cream was allowed to stand three minutes after the addition of dry sodium bicarbonate, hot water added at 180° and the mixture stirred for three minutes before filtering.

Any differences between the methods in dissolving some of the sediment were not considered of material importance. Results were satisfactorily duplicated by each method provided the cream was thoroughly mixed before sampling. F.W.B.

CHEESE

4. **Paying for Swiss Cheese on a Solids and Fat Basis.** ARTHUR B. EREK-
SON, Plymouth, Wisconsin. Natl. Butter and Cheese Jour., *37*,
10 : 48. October, 1946.

This method of payment is intended to give the producer of Swiss cheese
an incentive to make cheese with optimum amounts of fat and moisture. It
may also serve to protect the buyer who wishes to store cheese with high fat
and low moisture and is willing to pay for it. Cheese with more fat or less
moisture, or both, under this plan commands a proportionately higher price.
Cheese with less fat or more moisture or both is worth less. It is calculated
that cheese of optimum composition containing 61% dry matter, of which
45% is fat, has 0.2745 pounds of fat per pound of cheese. Cheese with the
same dry matter, of which 46% is fat, contains 0.2806 pounds of fat and
0.3294 pounds of solids-not-fat per pound of cheese. This amount of solids-
not-fat requires only 0.2695 pounds of fat to give the cheese the desired 45%
fat in the dry matter so there is an excess of 0.0111 pounds of fat for which
extra payment is needed. All of the value of the cheese price is assigned to
the dry matter. The difference between the cheese-solids price and the price
of butterfat in cream then is used to calculate the value of the amount of fat
over or under the optimum. These calculations have been made and tabu-
lated to show the value of a wide range of cheese compositions at the market
price for Swiss cheese of 33 cents per pound. In order to sell the cheese
beyond the primary market so that variations in purchase price will be fairly
covered, it is recommended that the average compositions of cheese in each
grade on the market be used to calculate the solids-plus-fat value of the
cheese in those grades. W.V.P.

5. **A Discussion on Cheese Standards of Identity.** RAYMOND MIOLLIS,
Natural Cheese Co., Maywood, Illinois. Natl. Butter and Cheese
Jour., *37*, 11 : 36. November, 1946.

The proposed "Standards of Identity for Cheese" is inadequate, espe-
cially for semi-hard and soft cheese of the imported type. The European
nomenclature can be disregarded because the names are meaningless and
obsolete for purposes of definition in the United States. The descriptions
of manufacturing processes in the proposed standards are vague, erroneous
and incomplete. Pasteurization of milk for all cheese should be compulsory
and without the 60-day optional curing alternative. The amount of milk
fat to be left in the cheese "should be left to the discretion of the cheese-
maker, and after the cheese is made every cheese should be branded . . .
with the butterfat content in multiples of five, followed by the sign +."
Semi-hard and soft cheese should have no moisture limits. W.V.P.

ICE CREAM

6. Research on Milk Solids-Not-Fat. B. I. Masurovsky. Ice Cream Trade Jour., *42*, 6 : 68. June, 1946.

Serum solids consist of milk protein, such as casein and albumen, which are used to build tissue and muscles. They also include the milk minerals to aid in the formation of bones and teeth. The average satisfactory serum solids in ice cream is about 11 per cent, depending upon the butterfat content of the mix. A high butterfat content requires less serum solids, but in no case should the combined milk solids exceed 22 per cent. Serum solids for ice cream are furnished by concentrated skim milk, plain or superheated; sweetened condensed skim milk; skim milk powder (spray process); and modified milk solids containing reduced quantities of sugar. Also, sweet buttermilk solids may be used. Skim milk used in the manufacture of skim milk solids should be tested for acidity, flavor, and sanitary quality.

W.H.M.

7. The Use of Processed Fruits, Nuts, and Other Flavors. E. C. Wetmore, Richardson Corp., Rochester, N. Y. Ice Cream Trade Jour., *42*, 6 : 56. June, 1946.

Some of the advantages of using processed fruits, nuts, and other flavors in ice cream are uniform flavor and color in the finished ice-cream, convenience in using, reduction of contamination of the ice cream because they are pasteurized after packing and sealing, and insurance of the maximum amount of flavor, because such fruits are usually selected and packed when they are at the right stage of ripeness. Nuts processed and packed in syrup retain their flavor and crispness in ice cream. Other flavors such as chocolate can also be obtained in sealed cans, and such flavors are usually uniform due to the ability of the processor to select the right blend of cocoa and chocolate liquor. W.H.M.

8. Powdered Ice Cream Mix. S. T. Coulter. Ice Cream Trade Jour., *42*, 5 : 42. May, 1946.

Dry ice cream mix is processed by condensing the milk-cream mixture which has been adjusted to the desired ratio of fat to solids-not-fat. Stabilizer dissolved in hot water, color, and sugar are then added to the condensed milk, and the mixture spray-dried to about 1 per cent moisture.

The capacity of the drier can be increased by adding only a portion of the sugar to the liquid mix prior to drying, and then blending the remainder of the sugar with the dry mix. Vegetable stabilizers are preferred to those of animal origin. Glycerol monosterate, egg yolk solids, powdered buttermilk, and sodium caseinate improve the whipping properties of dry ice cream

mix. The composition of dry ice cream mix varies, but the following is typical:

Fat ....27–29 per cent
M.S.N.F. ....26–27.5 per cent
Sugar ....40–44 per cent
Stabilizer ....0.6–1 per cent or more
Moisture ....1.2 per cent

The milk products are usually preheated to 190° F. for 5 minutes or more or to a higher temperature for a shorter holding period, to retard oxidative deterioration. Prompt cooling of the powder to 100° F. or less before it is packed is desirable. Cooling decreases cooked flavor and the rate of development of stale flavor. Low moisture and oxygen levels and storage of the powder at a low temperature (below 70° F.) are the principal means of retarding the development of stale flavor. W.H.M.

9. **Ice Cream Through Countless Ages.** ANONYMOUS. Southern Dairy Products Jour., *38*, 4 : 34. October, 1945.

Marco Polo during his journeys through the Orient in the fifteenth century became so fond of wines and sweetened water iced with snow from the mountains that he introduced the delicacy to his native Italy upon his return home. The Italians soon discovered that snow treated with saltpeter produced a mixture capable of freezing water ices.

About 1550 the Italians flavored the sweetened water with fruit juices and then substituted milk for water in the mixture. More and more cream was used. In the same century Catherine de Medici brought water ices into France and Italians set up water-ice shops in that country. Fancy molds were first introduced in 1774. The Germans introduced ice cream decorating as an art.

Ice cream was brought to England by Carlo Gatti from France, and King Charles I introduced it to his court. There are many conflicting stories as to who introduced ice cream to the United States. There are accounts of the serving of ice cream at the White House during the administrations of Andrew Jackson and James Madison and the product was advertised in New York and Baltimore 1786 to 1789 and in New Orleans in 1808.

The beginning of the wholesale manufacture of ice cream is credited to Jacob Fussell of Baltimore, Maryland, in 1850. The commercial manufacture spread to most of the other states during the remainder of the century.

Inventions of the Mojonnier milk tester in 1915, the ice cream overrun tester in 1917, the ice cream packaging machine in 1920, the Creamery Package 80-quart freezer in 1917, the ice cream cone in 1904, and the Eskimo Pie in 1921 were noteworthy contributions to the industry. Pennsylvania State College began instruction in the manufacture of ice cream in 1892 and Iowa State College in 1901. F.W.B.

10. The Use of Fruit Purees in Ice Cream. C. L. BEDFORD, Dept. of Horticulture, State College of Washington, Pullman, Washington. Ice Cream Trade Jour., *42*, 5 : 44. May, 1946.

Unsweetened fruit purees may be made by passing such fruits as raspberries, loganberries, boysenberries, youngberries, strawberries, Santa Rosa plums, and similar fruits through a tomato-juice extractor or a finisher such as is used to remove peel from cooked squash or pumpkin. The fruits should be pureed at 40–50° F. and then frozen. A deaerator may be used to remove air. This prevents changes in color and flavor during freezing, storage, and thawing. Peaches, apricots, and pears should be scalded or steamed for 4 to 5 minutes to prevent darkening when exposed to air.

Fruit purees may also be prepared by adding sugar or syrup to the puree. Two volumes of berries, sour cherries, plums, and strawberries may be frozen with 1 volume of 67% syrup; apricots and nectarines, 1 volume pulp to 1 volume of 50% syrup; and blackberries, Bing cherries, pears, 3 volumes pulp to 1 volume of 67% syrup.

Other types of fruit purees contain gelatin or pectin, and they may be used in making fruit ribbon, ripple, or marble ice cream, or in making a product developed by the Western Regional Research Laboratory called "Velva Fruit".

The formula for "Velva Fruit" is varied slightly for different fruits, but two typical formulas for 100 gallons of mix are given.

For unsugared fruit puree with high acid and low pectin content—such as raspberries, boysenberries, loganberries, youngberries, Santa Rosa plums, strawberries, and similar fruits:

Puree ....640 lbs.	610 lbs.	
Sucrose ....265 lbs.	170 lbs. (high-conversion corn	
Gelatin (275 Bloom) ...... 5 lbs. 13 ozs.	5 lbs. 13 ozs.	sirup—125 lbs.)
Water .... 60 lbs.	60 lbs.	

For fruits with low acid and high pectin content, such as unsugared apricots, pears, peaches, and similar fruits:

Puree ....680 lbs.
Sucrose ....225 lbs.
Gelatin (275 Bloom) .... 5 lbs. 13 ozs.
Water .... 60 lbs.
Citric acid .... 1 lb. 14 ozs.

In the high-acid fruits the soluble solids content should be about 37 to 38 per cent with about 1 part sugar to 2.4 parts of fruit puree. Corn sirup can be substituted for one-third of the sugar. It must be added in a 3 to 2 ratio to maintain the same sweetness.

In the low-acid fruits a soluble solids of 34 to 35 per cent and a sugar-fruit ratio of 3 to 1 is satisfactory.

In the preparation of the mix, the puree, sugar, and citric acid (if used) are mixed together until well dissolved. This should be kept relatively cool. The gelatin is mixed with ten times its weight of water and is heated to 170 to 180° F. to dissolve and "sterilize" it. During the addition of the gelatin sol the mix is stirred in order to prevent the formation of stringy gelatin in the mix. If a batch freezer is used, the gelatin sol can be stirred into the mix in the freezer before the refrigerant is turned on.   W.H.M.

11. Blower-Type Evaporators in Ice Cream Plants. C. H. MINSTER. Ice Cream Trade Jour., *42*, 8 : 34. August, 1946.

The blower type evaporator for ice cream plants has several advantages over the conventional unwieldy multiple-coil evaporator. It takes up less space, hardens ice cream faster, permits use of higher back pressures, lowers operation cost, is easily moved from one location to another, is easily defrosted, and requires little time for installation.

Two common troubles encountered in the operation of blower evaporators are frost on the coil and oil congealing in the float, surge drum, or coils. Most low-temperature units are equipped with a device for defrosting with calcium chloride brine. Sometimes these defrosters give trouble because of clogged brine spray, failure to close suction and liquid valves, weak brine, tendency to stop brine circulation before coils are completely defrosted, and the tendency to defrost only when frost has built up to a point where the circulation is reduced to a minimum. Heating the brine tended to offset these difficulties.

Some of the problems encountered with oil are: (1) Oil congeals in the float-valve orifice, preventing ammonia from entering the coils. Result: The evaporator would pump out and, with no refrigerant in it to absorb heat, all refrigeration would stop. (2) Oil, passing through the float-valve orifice with the float open, would congeal as soon as it hit the lower temperature of the float housing. Here it would build up and support the float in an open position. Result: Ammonia entered the coils in a steady stream and in quantities in excess of that required to maintain proper ammonia level. This ammonia would flood back on the compressor. (3) Oil would gradually accumulate and congeal in the bottom coils of the evaporator, resulting in reduced or completely shut off entrance of ammonia to coil, which further resulted in either refrigerant starvation or complete pump-out of the coils, thus slowing or stopping refrigeration. (4) The bottom coils became filled with fluid oil, resulting in reduced efficiency and heat transfer, and impediment of ammonia travel.

Remedies: (1) Maintain coils frost-free. (2) Constantly circulate air (from the room to be cooled) over the coils and back to the room. (3) Main-

tain the highest possible back pressure within the coils.   (4) Keep the entire unit oil-free.

Low-temperature blower type evaporators work better if installed in a separate room from the room to be refrigerated.   Some of the advantages of such an installation are: (1) Maintenance work on the unit can be more readily performed.   (2) Defrosting can be accomplished with plain water. (3) The unit can be warmed up while defrosting with a minimum rise in hardening room temperature.   (4) Oil can readily be drained from the unit.

<div align="right">W.H.M.</div>

12. **Ice Cream with Less Sugar.**   E. L. FOUTS, Dairy Technologist, Florida Experiment Station, Gainesville, Florida.   Southern Dairy Products Jour., *38*, 5 : 96.   November, 1945.

In 1943 the Florida Dairy Products Laboratory published Bulletin No. 393, entitled ''The Preparation and Use of Invert Sirup in the Manufacture of Ice Cream.''   In this bulletin, it was shown that it was possible to increase the sweetening power of cane or beet sugar by inversion.

It is possible to lower the sugar content of ice cream to 12% or even less by increasing the serum solids proportionately.   For example, if ice cream is being made with 10.5% fat, 11% serum solids, 14% sugar, and 0.25% stabilizer, the sugar can be reduced to 12% with a corresponding increase in serum solids from 11% to 13%, producing an ice cream which is quite satisfactory in sweetness and in other respects.   Trials have shown that the serum solids content can be increased to 13.5% if necessary.   The inversion of not more than 25% of the sugar also will tend to give increased sweetness. The use of invert sirup in excess of 25% of the total sugar lowers the freezing point of an ice cream mix so that it may be difficult to harden.

It has been proven that the degree of sweetness in ice cream is influenced by the amount of water in the ice cream mix.   This may be a partial explanation of why the sugar content can be reduced when the serum solids contribute additional milk sugar.   Actually, then, when the sugar content is reduced from 14 to 12% and the serum solids increased from 11 to 13%, the sucrose content is reduced 2% but in turn the lactose content is increased 1%.   If part of the sugar is inverted, the serum solids should be increased to maintain the same total solids in the mix, since the invert sugar sirup contains only about 70% sugar solids.

The additional serum solids seem to cover up or absorb vanilla flavor. It is suggested that a little extra vanilla be added when increasing the percentage of serum solids.   To reduce the sugar content of ice cream enough to be worthwhile without making other changes will result in a thin coarse-textured ice cream definitely lacking in sweetness and flavor.   F.W.B.

13. **Chocolate Ice Cream.** C. D. DAHLE, Pennsylvania State College. Southern Dairy Products Jour., *38*, 5: 28–30. November, 1945.

A sirup of two pounds of cocoa, two pounds of sugar and enough water to make a gallon of sirup is sufficient to flavor 10 gallons of finished ice cream. The reduction in the fat content of the mix from the addition of the sirup must be taken into account. The fresher the sirup the better is the flavor of the ice cream.

The flavor, body and texture of chocolate ice cream are improved by making a chocolate mix. The main drawback is that frequently the viscosity of the mix is so increased that cooling is difficult. The causes of high viscosity of the mix are: (1) stabilizer, (2) pressure, (3) acidity, (4) amount and kind of chocolate, (5) composition of mix, and (6) pasteurizing temperature.

Certain stabilizers cause more trouble than others. Reduce the amount used 20 to 30 per cent or more.

Single-valve homogenization will cause greater viscosity than "two-stage". The pressure on the single valve may be reduced from 2500 pounds to 1500 or less and in two-stage homogenization to 1500 and 500 pounds on the respective valves. The temperature of pasteurizing and homogenizing should be at least 160° F.

The acidity of the unflavored mix should be standardized and vary from about 0.15% for 8% M.S.N.F. to 0.20% for 12% M.S.N.F.

The more chocolate used the thicker and darker will be the product. The normal amount is 3–3½ per cent. The flavor is carried by the cocoa solids-not-fat and not the fat. The amount of each chocolate product needed to flavor 10 gallons of ice cream is about as follows: chocolate liquor (50% cocoa fat), 3.0 lbs.; blend (35% fat), 2.3 lbs.; cocoa (25% fat), 2.0 lbs.

Chocolate ice cream contains more sugar than vanilla ice cream to reduce the bitterness from the flavor. The more cocoa used, the more sugar is needed. A lower cocoa content may be compensated by a reduction in sugar to make the cocoa "taste stronger".

Chocolate is a relatively inexpensive flavor. In 1938, it was used in flavoring 16.36% of the ice cream and was surpassed only by vanilla, used in 51.26%.

Variegated chocolate ice cream may be made by adding a thick sweetened sirup to the ice cream as it is discharged from the freezer. The sirup may be purchased or made in the plant to contain 45–50% sugar, 1% stabilizer and 15% cocoa.

Sweetened slab chocolate may be melted at 105° F. and added to the ice cream as it comes from the freezer to give the ice cream a specky appearance. The colder the chocolate and the ice cream, the larger will be the pieces.

(The original article appeared in Serum Solids Digest and was reprinted by permission of Prestige Products Company, New York.) F.W.B.

14. **Frozen Food Packaging—A Preliminary Study of Cavity Ice.** C. I. SAYLES, W. A. GORTNER, AND FRANCES E. VOLZ, School of Nutrition, Cornell University, Ithaca, New York. Food Freezing, *1*, 11 : 430. September, 1946.

The term "cavity ice" is suggested to designate the formation of ice in the air spaces inside a package of frozen food stored in a cabinet. The condition is found even though water-vapor transmission is at a minimum because of protective covering. The condition arises from dehydration of the food within its protective covering, the resulting withdrawn moisture being deposited as ice crystals within the cavities of a loosely packed product like snap beans or on the surface of a solid product like meat. The authors attribute the phenomenon in part to radiant heat transfer of moisture from the food to the air within the package. Interposition of foil prevented the formation of cavity ice because of its radiant heat transfer barrier between the package and side wall plates. Maintenance of temperature uniformity is highly important in order that the temperature gradient within the package will be small, thus keeping down moisture abstraction from the food. Insulation of package was found to be effective in keeping down temperature fluctuation but at present would be prohibitive in cost. In general, cabinet design which would favor a higher humidity level than that commonly found would prevent moisture transferal. Also, keeping down the temperature change of refrigerated surface in cycling will aid materially. Seventeen illustrations and three diagrams.   L.M.D.

15. **Frozen Food Stores.** VINCENT M. RABUFFO. Ice Cream Trade Jour., *42*, 7 : 26. July, 1946.

The Ice Cream Trade Journal has made an ice cream sales survey in specialized frozen food stores in 17 eastern and midwest communities. It was found that the volume of sales ranged from 5 gallons to 300 gallons a week. All stores sold ice cream made by commerical manufacturers and only one store expressed a desire to make its own ice cream. Sixty per cent handled a deluxe high-fat ice cream, while 40% handled the regular commercial grade. Packaged ice cream was sold by 95% of the stores. The other 5% handled both bulk and packaged ice cream. Most of the stores were not interested in dipping ice cream. The price on deluxe ice cream ranged from 38 cents to 50 cents per pint, and standard grades averaged 30 cents per pint. Most of the deluxe grade sold for 45 cents per pint.

The price paid to manufacturers for the regular grade ranged from $1.25 to $1.60 per gallon, with most of them paying $1.50. These prices prevailed during July before any general advance went into effect. The wholesale price for the deluxe grade ranged from $2.00 to $2.85 per gallon.

Seventy-seven per cent of the stores handling the deluxe grade had a mark-up of 50% or more. It has been established that a good quality ice

cream at a good price can be sold by these stores. These mark-ups have been higher than those enjoyed by other retail outlets on ice cream. W.H.M.

PHYSIOLOGY

16. **The Relation of Temperature and the Thyroid to Mammalian Reproductive Physiology.** RALPH BOGART AND DENNIS T. MAYER, Missouri University, College of Agriculture, Columbia. Amer. Jour. Physiol., *147*, 2: 320–328. October, 1946.

High temperatures cause a marked lowering in the activity of the reproductive organs of rams.

Thyroxine or thyro-active proteins given to rams during periods of high temperature stimulate the reproductive organs and restore most of the reproductive activities to a level near that of the breeding season.

Changes in semen characteristics similar to those resulting from high environmental temperatures are induced during the breeding season by administration of thiouracil. Thyro-active materials counteract the detrimental effects of thiouracil upon reproduction.

Since the temperatures which will reduce fertility in the ram are below the temperature at which the testes normally function, and since thyro-active materials restore reproductive activity during periods of high temperature, it is concluded that temperature is not influencing reproductive physiology by its direct effect on the testes.

The stimulating effect of thyroxine during periods of high temperatures and the harmful effect of thiouracil administered during the normal breeding season suggest that the level of thyroid function influences the relative activity of the reproductive organs. D.E.

MISCELLANEOUS

17. **Styrofoam—A New Thermal Insulation.** O. R. McINTIRE AND D. W. McCUAIG, The Dow Chemical Company, Midland, Michigan. Refrigerating Engineering, *52*, 3: 217. September, 1946.

A polystyrene foam, "Styrofoam", now being produced in limited quantities, is an extremely light plastic structure composed of many small plastic bubbles, each bubble being completely enclosed to make the entire plastic structure impermeable. Styrofoam 103.7 has a K factor of 0.27 to 0.29 and cell sizes of 1/32 to 1/16 inch. Experimentally a product with aluminum powder incorporated in it has a K factor of 0.20 due to decreased infrared transmission. Styrofoam has in addition the following desirable factors: low specific gravity, low water absorption, high resistance to vapor diffusion, structural strength yet bendable, easily installed in sheets. Because it is made from polystyrene, a thermoplastic, Styrofoam begins to dis-

tort upon continuous exposure to temperatures above 180° F. but at low temperatures it becomes tougher and stronger. Installation procedure in commercial applications such as cold storage plants can be followed as for corkboard with the single precaution that asphalt temperature must be kept low enough (not over 175° F.) to prevent the Styrofoam from softening. Due to its very light weight it lends itself to the insulation of mobile equipment where the minimizing of weight is of prime importance. It should prove to be of exceptional value in the field of frozen foods transportation where the maximum of insulation effect is needed to hold low temperatures together with a maximum of goods weight and maximum of cargo space.

L.M.D.

ABSTRACTS OF LITERATURE

BACTERIOLOGY

18. **The Origin and Control of Thermoduric Organisms. Some Fundamental Phases.** DAVID LEVOWITZ, New Jersey Dairy Lab., New Brunswick, N. J. N. Y. State Assoc. Milk Sanit., Ann. Rpt., *19:* 219. 1945.

Thermoduric bacteria are present in milk freshly drawn from normal, healthy cattle. Research has not established whether poor health of cows raises their numbers. The cow does not affect the thermoduric content of milk enough to affect municipal milk standards. Bedding, manure, and feed dusts are sources of thermoduric bacteria. The sediment test would begin to mean something if straining of milk on the farm were eliminated. Contamination of milking machines with thermoduric bacteria may become important when machines are not handled properly.

Although thermoduric bacteria are not pathogens, they are associated with unclean equipment surfaces and should be held at minimum numbers. It is difficult to sterilize film-coated surfaces. High-temperature, short-time pasteurization cannot yield thermoduric counts as low as the holder method.

<div align="right">A.C.D.</div>

19. **Significance of Thermoduric and Thermophilic Bacteria in Milk and Their Control.** F. W. FABIAN, Division of Public Health, Michigan State College, East Lansing, Mich. Jour. Milk Technol., *9*, 5: 273–278. Sept.–Oct., 1946.

Thermophilic bacteria are more resistant than thermoduric organisms. They are distributed widely in feeds, grain, soil, cow hairs, manure, and improperly cleaned utensils. These organisms may contaminate the dairy plant from producers' milk and be propagated in the pasteurizing equipment, if it is not properly cleaned. These groups of organisms are not pathogenic. Their presence in appreciable numbers in milk indicates unsanitary practices both on the farm and in the milk plant. H.H.W.

20. **Heat Resistant Bacteria from an Unclean Milking Machine Invade the Udder of the Cow.** C. S. BRYAN, H. S. BRYAN, AND KARL MASON, Michigan Agricultural Experiment Station, East Lansing, Mich. Milk Plant Monthly, *35*, 8: 30–32. 1946.

The properly pasteurized milk from a small dairy increased during a 4-month period from its normal level of 15,000 to as high as 2,500,000 on some days. Despite careful plant cleaning and sanitizing, the high count continued. The source of the high count of heat-resistant bacteria was

found to be the milk of one of the seven producers. An unclean milking machine contributed heat-resistant bacteria to the milk, both directly during the milking process and indirectly by inoculating the cows' udders with these bacteria. The cows were free of the heat-resistant bacteria in periods varying from 1 to 4 months after the milk equipment was properly cleaned and sanitized. During this same period, the standard plate count of the properly pasteurized milk of this dairy decreased to its previous level of 10,000 to 25,000.   G.M.T.

21. **Thermal Death Range of Bacteria in Milk. A New Electric Sampling Device.** F. W. GILCREAS AND J. E. O'BRIEN, New York State Dept. of Health, Albany, N. Y. N. Y. State Assoc. Milk Sanit., Ann. Rpt., *19:* 237. 1945.

Study of the destruction of pathogenic bacteria by temperatures higher than now employed in the dairy industry is needed. Recently the Trumbull Electric Co. of Plainville, Conn., constructed a mechanism, based upon ideas of C. W. Weber, which makes possible laboratory pasteurization with accurate one-second holding intervals. The equipment is electrically activated and adds culture and withdraws samples by syringes. Tests were made on one ordinary *Escherichia coli* culture and two heat-resistant strains from the U. S. Public Health Service. It was concluded that the thermal death point for *E. coli* is not constant, and "therefore only a thermal death range based on results of repeated tests with many cultures can be determined."
                                                                A.C.D.

22. **The Survival of Staphylococci Food Poisoning Strain in the Gut and Excreta of the House Fly.** SARAH MOOREHEAD AND HARRY WEISER, Dept. of Bacteriology, Ohio State University, Columbus, Ohio. Jour. Milk Technol., *9*, 5: 253–259. Sept.–Oct., 1946.

A food-poisoning strain of staphylococcus, *Staphylococcus aureus* 611, suspended in a dilute sucrose solution, was fed to 900 "cultured" houseflies, *Musca domestica.* The test organism was recovered from the digestive tract in some of the flies periodically through 8 days.

Staphylococci not of a food-poisoning strain were isolated from the digestive tract of 10 of the 50 wild-caught flies examined. This indicates that staphylococci may be commonly carried by flies.

Musca domestica may serve as a reservoir host for *Staphylococcus aureus* 611, and under suitable conditions the fly may initiate or augment a food-poisoning outbreak by spreading staphylococci from infected handlers or dirty equipment to food and from contaminated supplies to good foodstuffs which are favorable for enterotoxin production. The organism may survive in the digestive tract of the housefly several days after contamination and be deposited on food even after the carrier source has been removed or after the fly has sought a new feeding location.   H.H.W.

23. **The Influence of Surface Active Cationic Germicides on the Bacterial Population of Milk.** ADRIEN S. DUBOIS AND DIANA D. DIBBLEE, Onyx Oil and Chemical Co., Jersey City, N. J. Jour. Milk Technol., *9*, 5 : 260–268. Sept.–Oct., 1946.

Alkyldimethylbenzylammonium chloride did not influence the bacterial counts of raw or pasteurized milk at concentrations ranging from 1 : 500 to 1 : 25,000. The higher concentrations had an inhibitory effect on the growth of Gram-positive acid-producing organisms but did not affect the Gram-negative bacteria. When this compound was used in lower concentrations, no such effect was noted. A chemical method for the estimation of surface-active cationic germicides in milk and a qualitative test for their detection in solutions are described.   H.H.W.

BUTTER

24. **Experiments on the Packing and Storage of Butter. Part V. The Effect of the Temperature-Level of Storage on the Keeping Quality.** C. R. BARNICOAT, Dairy Res. Inst., Palmerston North, New Zealand. New Zeal. Jour. Sci. and Technol., *27*A, 4: 343–348. Dec., 1945.

At storage temperatures between 14° and 60° F., the average rate of deterioration of well-made sweet cream butter, as measured by decrease in the score for flavor, was related directly to both time and temperature. The average rate of loss in score increased by about 0.15 point per week for each 10° F. rise in temperature above 14° F.

The slight advantage obtained by storage at −5° F. instead of 14° F. was not warranted by the extra cost.   W.C.F.

25. **Some Experiments on the Use of Parchfoil and Pliofilm for the Wrapping of Butter in *Pinus radiata* Boxes.** F. H. McDOWALL, Dairy Res. Inst., Palmerston North, New Zealand. New Zeal. Jour. Sci. and Technol., *27*A, 4: 303–308. Dec., 1945.

A strong wood flavor developed within 10 days in butter wrapped in parchment and packed in boxes made of *Pinus radiata*. Wrapping in parchfoil (aluminum foil between parchment sheets) prevented the development of primrose color on the surface and wood taint in the butter up to 2 years, except where the wrappers joined. Pliofilm wrapping prevented the color defect but permitted the absorption of the flavor within 6 months. The treatment of the box with pliowax did not prevent the taint. Tensilized pliofilm was not satisfactory. Little trouble with mold growth was encountered, but under commercial conditions molds might appear under the pliofilm.   W.C.F.

26. **Measurement of the Gas Content of Concentrated Butter and Other Fat Products.** G. L. HILLS AND J. CONOCHIE (Div. Indust. Chem.). Austral. Council Sci. & Indus. Res. Jour., *18*, 4: 366–372. Nov., 1945.

A modification of the method of Rahn and Mohr is described.

W.C.F.

27. **The Manufacture of Dry Butterfat and of "Butter Concentrated Hardened."** W. J. WILEY AND G. W. COOMBS (Dairy Research Section). Austral. Council Sci. & Indus. Res. Jour., *19*, 1: 140–146. Feb., 1946.

Methods for large-scale production are described.

W.C.F.

28. **Experiments on the Manufacture and Storage of Ghee.** C. R. BARNICOAT, Dairy Res. Inst., Palmerston North, New Zealand. New Zeal. Jour. Sci. and Technol., *27*A, 4: 309–319. Dec., 1945.

Attempts to produce ghee from cow butterfat yielded a product resembling Indian ghee but not entirely characteristic. Dry New Zealand butterfat was found to be acceptable to the Indian trade. These products when canned kept fairly well over 9 years of storage at 40° F.   W.C.F.

CHEESE

29. **The Effect of Hydraulic Pressing on Cheese Texture.** H. R. WHITEHEAD, Dairy Res. Inst., Palmerston North, and L. J. JONES, Dairy Div., Dept. of Agr., Wellington, New Zealand. New Zeal. Jour. Sci. and Technol., *27*A, 5: 406–410. Feb., 1946.

When pressing cheddar cheese by hand-screw presses was compared with pressing by hydraulic presses, use of the latter almost eliminated "mechanical" openness in normal cheese of good quality but did not influence "slit" openness which may develop later.   W.C.F.

CHEMISTRY

30. **Surface Chemistry in Chemical Cleaners.** GEORGE J. LEHN, Turco Products, Inc. Milk Plant Monthly, *35*, 7: 50–53. 1946.

Much time is spent in eliminating soil, such as film, scale, rust, and casein accumulations, from surfaces of dairy equipment. New knowledge of cleaners points more toward surface chemistry, which involves destroying the bond holding the soil to the surface. Wetting action brings cleaning solutions into close contact with oily, fatty, adhesive films, in order that soil may be dislodged more easily. Better wetting is made possible by the

control of surface and interfacial tensions. Cleaning a surface involves wetting, emulsifying, saponification, and solvent action. Carefully formulated cleaners are buffered so that their cleaning energy will be retained. Also, properly formulated cleaners condition and control the precipitation of minerals in hard water, thus assuring free and complete rinsing. Applications of surface chemistry to cleaners should save enormous amounts of production time and costs in dairy sanitation.　　　　　G.M.T.

31. The New Non-Chlorine Disinfectants. D. H. JACOBSON. Ice Cream Field, *48*, 4: 70. Oct., 1946.

Quaternary compounds are compared with chlorine compounds as germicides. The following advantages of quaternary compounds in dairy plants are listed: (a) non-corrosive on common metals, (b) non-toxic, (c) stable under heat and over long periods of time in common dilutions, (d) non-irritating to the skin, (e) colorless and odorless in common dilutions, (f) rapid wetting and penetrating action, (g) relatively non-selective towards various types of bacteria, (h) surfaces may be rendered bacteriostatic for some time after treatment, (i) not affected by hard water salts.

The organization of the "National Sanitation Foundation" in 1945 in the School of Public Health at the University of Michigan is mentioned. This organization is supported by the industry, and expectations are that the information required for developing the use of the products in dairy plants will be supplied. Brief mention is made of some experimental results already published by other workers.　　　　　W.C.C.

32. The Use of Quaternary Ammonium Compounds in the Dairy Industry. C. A. LAWRENCE, Winthrop Chemical Co., Rensselaer, N. Y. N. Y. State Assoc. Milk Sanit., Ann. Rpt., *19:* 177. 1945.

The quaternary ammonium compounds are surface-active agents consisting of two parts, a hydrophilic group and a lipophilic group. Since the lipophilic group is charged positively, these compounds also are known as cationic sterilizers. The wetting action, degree of detergency, and lowering of surface tension do not in themselves determine sterilizing action. The cationic sterilizers are effective for both Gram-positive and Gram-negative bacteria. These are most effective in alkaline solutions.

The cationic sterilizers are non-corrosive, non-toxic, odorless and non-irritating to the skin. They have high phenol coefficients. High concentrations of organic matter reduce their efficiencies. Soap and other anionic detergents also reduce their efficiency. These cationic compounds (alkyldimethylbenzylammonium chloride known as Zephirol, Zephiran, and Roccal was given as an example) have been found to be effective in sterilizing dairy equipment.　　　　　A.C.D.

33. **Higher Fatty Acid Derivatives of Proteins.** W. G. GORDON, A. E. BROWN, AND R. W. JACKSON. Eastern Regional Res. Lab., U. S. Dept. of Agr., Philadelphia 18, Pa. Indus. and Engin. Chem., Indus. Ed., *38*, 12 : 1239–1242. Dec., 1946.

Casein and other proteins were modified by preparing a series of novel fatty-acid derivatives by the reaction of acid chlorides with the proteins dissolved in aqueous alkali. The procedure developed gave derivatives of casein which were acylated to the extent of approximately 20% by substituent groups ranging from caprylyl to stearoyl. The physical and chemical properties of palmitoyl casein are discussed. The acylated products show reduced affinity for water and altered solubilities.   B.H.W.

34. **Plastic Properties of Higher Fatty Acid Derivatives of Proteins.** W. G. GORDON, A. E. BROWN, C. M. McGRORY, AND E. C. GALL, Eastern Regional Res. Lab., U. S. Dept. of Agr., Philadelphia 18, Pa. Indus. and Engin. Chem., Indus. Ed., *38*, 12 : 1243–1245. Dec., 1946.

Data on the water absorption and tensile and flexural strengths of molded test specimens of the higher fatty-acid derivatives of casein and other proteins are compared with similar data for casein hardened with formaldehyde. The acylated casein molding powders flow well in small positive type molds, and the molded pieces do not require further treatment with formaldehyde to yield finished articles. The molded specimens were light to dark yellow and translucent, with many almost transparent.   B.H.W.

CONCENTRATED AND DRY MILK; BY–PRODUCTS

35. **Keeping Qualities of Whole Milk Powder and Oatgum Mixes.** H. A. BENDIXEN, Division of Dairy Husbandry, Washington State College, Pullman, Wash. Ice Cream Field, *48*, 4 : 68. Oct., 1946.

Comparisons of oatgum and sodium alginate as stabilizers for ice cream are reported. Whipping ability, flavor, and texture were similar when 0.5% of the former and 0.22% of the latter were used. A gravimetric method of determining solubility of milk powder, developed at the Washington Experimental Station, is mentioned.   W.C.C.

36. **The Keeping Quality of Australian Milk Powders.** C. C. THIEL AND E. G. PONT (Dairy Research Section). Austral. Council Sci. & Indus. Res., *18*, 4 : 373–390. Nov., 1945.

Gas packing did not improve materially the storage life of skim milk powders but greatly increased that of whole milk powders. Because of leakage of cans, the results of gas packing on a commercial scale often were

nullified. The influence of different storage temperatures tried, 15, 30, and 37° C., was not marked. No correlation was observed between bacterial counts and the initial or final quality of the powder. Working fresh butter-fat into stored skim milk powder gave a product no better than the stored, gas-packed, whole milk powder.   W.C.F.

37. **Studies on Compressed Whole Milk Powder.** C. C. THIEL (Dairy Research Section). Austral. Council Sci. & Indus. Res., *18*, 4: 391–406. Nov., 1945.

The compression of spray-dried whole milk powder to a density of 1.15 to 1.2 reduced the interstitial oxygen as effectively as did the usual gas pack-ing in cans, but neither cellophane nor waxed paper wrapping prevented the uptake of atmospheric oxygen or moisture. Blocks made of the milk powder containing 20% of cane sugar remained more friable and kept better than blocks made up of milk powder alone. Added vanillin improved the keeping quality of the milk powder in blocks.   W.C.F.

38. **Cultured Dairy Products, Production and Quality Control, Part I . . . . Buttermilk.** S. M. MANN, General Biochemicals, Inc., Chagrin Falls, Ohio. Milk Plant Monthly, *35*, 8: 26–29. 1946.

A brief review of literature on the development of dairy cultures and on some of the salient facts pertaining to the development of a high-quality cultured buttermilk is given. The cardinal principles to observe in the handling of milk cultures are: 1. Use best-quality milk; 2. Work cleanly and carefully in clean, draft-free surroundings; 3. Use clean utensils, preferably those which have been sterilized or rinsed in chlorine (20–30 p.p.m.) solu-tions; 4. Strictly observe sterilization, pasteurization, incubation, and cool-ing temperatures.

The production of high-quality cultured buttermilk involves: (a) pas-teurizing milk at 185 to 190° F. for 30 minutes; (b) cooling and setting to 70° F.; (c) adding 4 gal. of 20% cream to 100 gal. skim milk; (d) adding 1 to 2% starter; (e) setting for 16 hours at 70°; (f) salting at the rate of 0.5 oz. per 10 gal., and adding highly colored butter granules if desired, after which the cultured milk is churned at high speed 5 to 10 minutes; (g) drawing off the churned cultured buttermilk into a coil vat and cooling to below 40° F.   G.M.T.

DISEASES

39. **Mastitis Prevention.** I. E. PARKIN, Pennsylvania State College, State College, Pa. N. Y. State Assoc. Milk Sanit., Ann. Rpt., *19:* 187. 1945.

Mastitis can be eliminated almost entirely from our dairy herds, even though research at Pennsylvania State College has shown that mastitis-pro-

ducing bacteria are present in the udder of practically every cow. The problem is primarily one of best herd-management practices.

Factors of major importance are good barns, well-bedded stalls of ample size, feeds of high vitamin A potency, prepartum milking of cows whose udders are inflamed, and the new managed-milking program. One of the greatest preventatives of mastitis is the use of managed-milking practices, which are described briefly. Several instances of marked improvement of mastitis by the managed-milking program were cited.   A.C.D.

HERD MANAGEMENT

40. The Effect of the Level of Stimulus Applied by the Pulsator on the Rate of Machine Milking. W. G. WHITTLESTON, Animal Res. Sta., Dept. of Agr., Ruakura, New Zealand. New Zeal. Jour. Sci. and Technol., *27*A, 5: 445–450. Feb., 1946.

Under otherwise normal conditions of machine milking, the application of a greatly reduced pulsator stimulus had no effect on the milking rate. Once the milk flow starts with the pulsator operating, it will continue if the pulsator is stopped. Normal milk letdown is not obtained, however, if an attempt is made to milk without the pulsator when the teat-cups are applied.
                                                              W.C.F.

ICE CREAM

41. How Golden State Cuts-Wraps Slices. GEO. D. AMERDING, Mojonnier Bros. Co. Ice Cream Field, *48*, 4: 76. Oct., 1946.

The cut-wrap slice machine is claimed to give the best and most popular individual serving of ice cream. The machine will produce 5,000 slices per hour, which is equal to 200 gallons of ice cream if cut seven slices to the quart. With waxed paper costing $0.13 per lb., waxed liners for cartons will cost $0.241 per M. Using the cut-wrap slice machine, labor costs for slicing one gallon of ice cream would be about 2.5 cents. The cost of the individual slice of ice cream is lower than any other individual package so far introduced.   W.C.C.

42. Manufacture of Dry Ice Cream Mix. S. T. COULTER, Dairy Division, University of Minnesota, St. Paul, Minn. Milk Plant Monthly, *35*, 7: 84–85. 1946. Also Ice Cream Field, *48*, 4: 56. Oct., 1946.

Dry ice cream mix developed during the war has some advantages over liquid mix for ice cream manufacturers who buy mix. Transportation costs are lower, refrigeration unnecessary, and the mix is less perishable. Dry ice cream mix often is subject to oxidation during storage, the rate of oxidation depending upon such factors as freshness of dairy products used, degree of

preheating of the milk, the presence of metallic catalysts, and temperature of storage. The moisture level of the dry mix seems to be a persistent factor influencing staling. Keeping quality of the dry mix may be improved by reducing the moisture level as low as 1%, and avoiding storage at unduly high temperatures. Composition of dry ice cream mixes and steps in processing the dry ice cream mix are given. G.M.T.

43. **Analysis of Stabilizers.** C. D. DAHLE, Pennsylvania State College, State College, Pa. Ice Cream Field, *48*, 4: 62. Oct., 1946.

Gelatin, sodium alginate, locust bean (carob gum), Irish moss, sodium carboxymethyl cellulose, oatgum, pectin, Karaya, and Psyllium seed husks (ground) as stabilizers in ice cream are discussed. Because of the shortage of certain stabilizers during the war, several "mixed" stabilizers were placed on the market. These often contain various sugars which aid in dispersing the stabilizing agent and also may contain one or more of the following: sodium bicarbonate, sodium citrate, whey powder, milk solids dextrines, and, possibly, emulsifying agents. Emulsifying agents such as glycerol mono-stearate, sodium mono-palmitate, and sorbitan monostearate are mentioned briefly as aids to the whipping ability of ice cream mixes. W.C.C.

44. **Flavors and Fruits from Brazil.** H. A. CARDINELL, Horticultural Section, AND P. S. LUCAS, Dairy Section, Michigan State College. Ice Cream Field, *48*, 4: 58. Oct., 1946.

Results of experiments in which certain Brazilian fruits were tried as flavors in ice cream are given. The juice of the passion flower fruit, known in Brazil as "maracuja," has a sour taste somewhat similar to orange juice; it was better suited for ices or sherbets than for ice cream. Juice of the cashew plant gave a pleasing, nut-like flavor which blended well with ice cream containing nut meats. The authors stress the desirability of using certain of these foreign fruits in ice cream. W.C.C.

45. **Nuts for Your Winter Ice Cream.** W. J. CAULFIELD AND C. A. IVERSON, Department of Dairy Industry, Iowa State College, Ames, Iowa. Ice Cream Field, *48*, 4: 84. Oct., 1946.

The use of nuts in ice cream enhances the nutritive value of ice cream, since nuts are good sources of fat, protein, vitamins (A, B, and G), and minerals (calcium, phosphorus, iron, and copper). Selection of nuts with good flavor, prevention of deterioration, and development of crispness as a result of proper roasting are important considerations, but the question of bacterial contamination of ice cream through the use of nut meats deserves more consideration than it has received in the past. Small manufacturers can use prepared nuts to advantage, whereas the large manufacturers ordinarily find it advantageous to roast the nuts in their own plants.

Directions are given for preparing the following nuts for use in ice cream: almonds, cashews, hazel nuts, peanuts, pecans, pinions or pignolias, pistachios, black walnuts and English walnuts. Roasting procedures cannot be used according to time and temperature charts. Frequent examination during roasting is necessary to assure that the nuts will be light brown in color throughout as well as brittle and crisp when finished. The nuts should be chopped after roasting and 6 to 8 lbs. of nuts and 1 oz. of salt mixed with 1 lb. of butter previously heated to 330° F. (160° C.). They may be used immediately or stored in a closed container in the hardening room. Use 3 to 5 lbs. of buttered nuts per 10 gal. of ice cream.   W.C.C.

46. **Some New Facts on Ice Cream in Super-Markets.** R. W. MUELLER, Associate Editor of Progressive Grocer. Ice Cream Field, *48*, 4: 32. Oct., 1946. (Reprinted from Progressive Grocer.)

A recent survey of super-markets in the Los Angeles area shows that ice cream occupies only 0.88% of store display facilities but represents 2.02% of total store volume and brings 4.7% of total dollar margin. This same survey shows that the rest of the frozen food line occupies 1.96% of total display space, represents 4.56% of total store volume and 7.14% of the total dollar margin. Ice cream represents 30% of the total frozen food department and equals 40% of the department's gross.

Ice cream sales now are mostly in pints and quarts. With larger frozen food storage compartments in household refrigerators, food merchants anticipate the sale of larger packages, such as half-gallon and gallon sizes.

W.C.C.

47. **What to Do About Ice Cream Cabinets.** EDWARD L. KOEPENICK, Ex-Secretary, National Conference of Ice Cream Industries. Ice Cream Field, *48*, 4: 112. Oct., 1946.

Certain unfair trade practices in connection with credits, repairs, advertising, etc., often used in the ice cream industry as a means of distributing ice cream cabinets and controlling ice cream sales, are discussed. Mention is made of the California Agricultural Code which prohibits these unfair trade practices. The author advocates Federal legislation designed to abolish such evils.   W.C.C.

48. **Layout of Market to Feature Ice Cream and Frozen Foods.** BEMAN FAST, Store Planning and Market Fixture Division, Weber Showcase and Fixture Co., Inc., Los Angeles, Calif. Ice Cream Field, *48*, 4: 34. Oct., 1946.

The recently modernized Hollywood Ranch Market is described and the subsequent increase in sales noted. Self-service, glass-topped cabinets which

replaced blind storage cabinets have resulted in a 60% increase in ice cream sales and have doubled sales in some departments.   W.C.C.

49. Sale of Ice Cream by Weight. ANONYMOUS. Ice Cream Trade Jour., *42*, 8 : 32, 83. Aug., 1946.

The International Association of Ice Cream Manufacturers is opposed to the sale of ice cream by weight. Reasons for this stand are:

(1) The most expensive ingredient in ice cream, namely butterfat, is the lightest in weight.

(2) Although, proportionately, ice cream contains very much less air than does angel food cake, air in the proper quantities is just as important, and there is no more reason to sell ice cream by weight than there is to sell angel food cake by weight.

(3) In the proposed Food and Drug Administration standard for ice cream, the product must be agitated during freezing to avoid formation of a solid brick similar to a block of ice. The air in ice cream gives an insulating effect and makes ice cream melt more easily in the mouth.

(4) The most expensive ingredient of ice cream, butterfat, is lightest (7.76 lbs. per gal.), while its cheapest ingredient, invert sugar sirup, is heaviest (10 lbs. per gal.).

(5) A high-grade rich ice cream which would weigh not more than 4.6 lbs. or 4.7 lbs. per gal. may be made. A very cheap grade of ice cream, one poorer in almost all of the qualities that make a good ice cream, may be made to weigh 5.5 or 6 lbs. to the gallon.

(6) Most of the almost 200 different flavors which have been recorded for ice cream would have a different weight.

(7) More ice cream is sold at the soda fountain, in restaurants as individual servings or in ice cream cones than is sold to be taken home. It is entirely impractical to make sales of this kind by weight, even if it seemed desirable to do so.   W.H.M.

MILK

50. Post War Milk Bottle. V. L. HALL, Glass Container Manufacturers Institute, New York, N. Y. Jour. Milk Technol., *9*, 6 : 336–338. Nov.–Dec., 1946.

The square milk bottle gradually is being introduced into the dairy industry. In the dairy plant, the washing and handling of the square bottle is just as satisfactory as for the round bottle. A case of round bottles occupies 47.5% greater area than the square bottles; a 6-ft. household refrigerator will hold 12 square bottles in the area formerly occupied by 8 round bottles. Moreover, the square container permits the retail grocer to do a better job of packing groceries in bags and in shopping carts.   H.H.W.

51. Strainer Pad Control of Milk Quality. C. B. A. BRYANT, Johnson and Johnson Co., Chicago, Ill. N. Y. State Assoc. Milk Sanit., Ann. Rpt., *19*: 199. 1945.

Observations on sources of sediment were made during field demonstrations on farms all over the United States. A clean cow and a clean milk can are essential. Milk-can covers are often in dusty place when not in use. About 40% of all water on the farm contains considerable sediment. Half of the farmers do not put the cotton disk in the strainer correctly. Cotton pads commonly distort during use. Single gauze-faced disks should be placed in the strainer with the cotton side up. Strainers are often jammed and disks loosen. The strainer should be rinsed with water before a new disk is inserted. Milking machine teat cups frequently draw up bedding.

New York State experiences have shown several things. Dairies use 8-inch cotton disks when 5.5- or 6-inch disks would do, and cost about half as much. Some 85% of the strainers were not good. A fluid milk plant was selected for demonstration. Each farmer demonstrated correctly how to put the 6-inch disk into the strainer. Good strainers were installed. Emphasis was placed upon sanitary methods rather than *cleaning milk*. Excellent farm cooperation was obtained. Original strainers and used cotton disks were obtained for exhibition. After proper farm instruction had been given and correct equipment used, results showed definite improvement in sanitary procedures and milk quality.   A.C.D.

52. Laboratory Tests in the Control of Milk Supplies. EDITORIAL. Amer. Jour. Pub. Health, *36*, 11: 1309–1310. 1946.

This editorial concerns the review article by Dr. A. H. Robertson in the same number of the American Journal of Public Health. In part, the editorial states: "Dr. Robertson's specific recommendations with regard to Plan B (the Connecticut State Department of Health milk program) deal with legal and administrative problems which are of purely local interest to the State of Connecticut and involve no changes in laboratory procedure. With respect to Plan A (the U. S. Public Health Service Ordinance and Code), on the other hand, he makes three important recommendations with regard to testing technique: the routine testing of all samples of allegedly pasteurized milk by the use of the phosphatase test and the coliform test; the routine examination of all samples of pasteurized milk by the microscopic count; and use of the microscopic count for samples of raw milk from sources which fail to comply, especially when field inspectional methods fail to disclose the cause for high counts or short reduction times.

This is not a matter to be settled by any one expert, as Dr. Robertson would admit; but he has marshalled evidence which makes an impressive case against sole reliance on the laboratory tests included in Plan A. There is a clear challenge here to the experts in this field—and particularly to the

experts of the U. S. Public Health Service—to seek some common ground of agreement which can harmonize or combine the values of both Plan A and Plan B. We would urge that the procedure employed by water bacteriologists in their field be applied in this related area. This procedure involves the setting up of cooperative studies in which workers in various laboratories employ several alternative standardized procedures and compare results, with regard to the significance of results obtained in the examination of a considerable series of actual field samples and the time involved in each procedure. . . . Only by such a cooperative study, in which advocates of both Plan A and Plan B participate, can the solution be found of a difference of opinion which may otherwise place serious obstacles in the way of an effective system of milk control.''   M.W.Y.

53. **Laboratory Procedures in Sanitary Milk Control.** A. H. ROBERTSON, Director of State Food Laboratory, Department of Agriculture and Markets, Albany, N. Y. Amer. Jour. Pub. Health, *36*, 11: 1245–1259. 1946.

The author prepared this special review at the request of the Editorial Board. He concludes ''Under either the U. S. Public Health Service Ordinance and Code (Plan A) or under the Connecticut State Department of Health Program (Plan B), the fundamental objectives of maintaining the highest possible assurance of safety and continuous conformance to the standards for low count milk have not been as fully achieved as might be expected.

''Plan A would be improved by:

''1. Requiring the routine testing of all samples of allegedly pasteurized milk using the phosphatase test and the coliform test.

''2. Requiring a microscopic examination of all raw samples which fail to comply, especially when field inspectional methods fail to disclose the cause for high counts or short reduction times.

''3. Requiring routinely a microscopic examination of all pasteurized samples to determine whether or not high-count samples escape detection by the plate method.

''4. Permitting the use, where determinations have been checked periodically and found satisfactory, of routine plant reports on inspections and analyses of samples by licensed purchasing agencies in lieu of official inspections and analyses.

''Plan B would be improved by:

''1. Securing proper legislation fixing, or allowing the Dairy and Food Commissioner to fix by official order, standards for bacterial density in terms of results by methods which are to be used officially for the determination of compliance with the statute.

"2. Providing for more frequent routine inspections and examinations of samples, with prompt repeat inspections and repeat examinations of samples in cases of non-compliance until continuous conformance can be reasonably assured."   M.W.Y.

54. What the Dairy Industry Expects of the Sanitarian. C. C. HADLEY, Indiana Dairy Products Assn., Inc. Milk Plant Monthly, *35*, 8: 24–25, 56. 1946.

A good milk sanitarian should be a teacher, diplomat, and administrator, thus exhibiting common sense, sound judgment, diplomacy and practicability, and radiating a pleasing personality through good health, energy, unquestioned integrity and enthusiasm for his work. A health sanitarian is expected to be an ambassador of good will for sanitation programs; to make proper contact with the manager's office prior to inspection; to use gentlemanly procedures in calling the management's attention to existing conditions; to be consistent month after month, not overlooking recommendations made on the previous visit; to use judgment on big versus little things; to refrain from peddling information from plant to plant concerning conditions; to be loyal to his superior; and to be well-trained for his job. Attention is called to a report appearing in the JOURNAL OF DAIRY SCIENCE, August, 1944, concerning courses which should aid in the training of a dairy sanitarian.   G.M.T

MISCELLANEOUS

55. Dairy Waste Saving and Disposal. WILLIAM A. DEAN, JR., Bowman Dairy Co., Chicago; Chairman, Task Committee on Dairy Waste Disposal. Milk Indus. Found. Assoc. Bul., *39*, 2: 30–42. Dec., 1946.

The problem of waste is serious because litigation and punitive expense frequently result from overloading existing sewage disposal facilities. Systems of undue size may be required because of excessive quantities of waste. Without change in equipment, one plant reduced daily waste from a B.O.D. (biological oxygen demand) of 260.5 lbs. or a population equivalent of 1,580 to a B.O.D. of 73.4 lbs. or a population equivalent of only 450. Many causes of waste are listed. The following recommendations are made: drip savers; pre-rinses; electronic level controls on tanks and troughs subject to overflow; improved maintenance of pumps, fittings, valves, etc.; elimination of foam in separation; adequate storage tanks for whey, buttermilk, and skim milk; standby pumps where needed to handle these products if regular pumps fail; special entrainment separators and other controls on vacuum pans. Special emphasis is placed upon preventing dilution of wash waters and necessary wastes with condenser and clear waters.

Every plant seemingly in need of additional waste treatment facilities should make sure that losses are reduced to a minimum. Use of continuous proportionate sampling device and determinations of turbidity and B.O.D. are recommended. E.F.G.

56. **The Effect of Dairy Factory Drainage Upon the Quality of Streams in Taranaki.** P. O. VEALE, Taranaki Service Laboratories, New Zealand. New Zeal. Jour. Sci. and Technol., *27*B, 4: 282–301. Jan., 1946.

With reasonable dilution available, the discharge of dairy plant wastes into a well-oxygenated stream had only a temporary effect upon the quality of the water. The greater the dilution, the shorter was the distance downstream to where effects were no longer evident. W.C.F.

57. **The Oregon Program of Licensing Cheese-Makers, Butter-Makers and Pasteurizer Operators.** G. H. WILSTER, Oregon State College, Corvallis, Oregon. Jour. Milk Technol., *9*, 6: 317–321, 328. Nov.–Dec., 1946.

The licensing of cheese-makers, butter-makers, and pasteurizer operators is an important step in Oregon's dairy products quality-improvement program. The compulsory milk and cream grading law enacted in 1937 provides for the grading of these products when received at factories and creameries, licensing of persons who are doing the grading, and payment for the milk and cream in accordance with quality. Inferior quality of milk and cream must be denatured by adding red coloring matter and must be tagged, and returned to place of origin. In 1939, an amendment requiring the issuance of licenses upon passing of an examination for butter- and cheese-makers made the act much more effective.

A new law, passed in 1945, governs the pasteurization of milk and milk products and licensing of pasteurizer operators. The ultimate goal of these standards is: To raise the general standard of proficiency of the Oregon butter-makers and cheese-makers, to manufacture the highest-quality cheese and butter of correct composition, to increase the demand for Oregon cheese and butter in out-of-state markets, and to increase the return to the Oregon dairy farmers. H.H.W.

58. **Insect Control in Dairy Plants.** GEORGE E. GOULD, Purdue University. Milk Plant Monthly, *35*, 7: 38, 54–55. 1946.

Control of dairy farm and dairy plant insects is essential to the manufacture of quality dairy products. Insect control is linked closely with plant sanitation and is actually a part of that program. Insects are attracted to dairy plants because of milk and milk products, although some insects are

attracted because of lights. The first and most important step in fly control is sanitation. Thorough daily cleaning of all equipment used is imperative. Daily burning and disposal of refuse is essential. Sixteen-mesh screening will check entry of flies, mosquitoes, gnats, and other small insects into the plant. The use of DDT should not be considered as the only necessary control measure, but as supplementary to sanitation. Specific directions are given for applying DDT. Descriptions are given of the various roaches infesting dairy plants. Sodium fluoride alone, or diluted with 25% pyrethrum powder, is a standard roach powder. Sprays have never been entirely successful in roach control. Sanitation is an important part of roach control. G.M.T.

ABSTRACTS OF LITERATURE
BACTERIOLOGY

59. **Comparison of Resazurin Test with Methylene Blue.** G. OKULITCH, R. MILLARD, AND O. FLEMING. Canad. Dairy and Ice Cream Jour., *25*, 11 : 35. Nov., 1946.

Milk can be graded effectively by means of the resazurin test using a single color standard, mauve pink no. 12 or Munsell notation P.R.P. 7/8, and making readings at 1 and 3 hr. The resazurin test and the methylene blue test agree well in selecting poor-quality raw milk. The correlation of resazurin and methylene blue is approximately 80%. Both the standard plate count and the direct microscopic count support the resazurin test in more cases than they do the methylene blue test. A high percentage of samples that test 3 hr. or more with resazurin have counts of less than 200,000 per ml. by the plate method and less than 100,000 by the direct microscopic procedure. A resazurin mauve pink of 3 hr. or more represents as good-quality milk as a 6.5-hr. methylene blue test. A greater number of physiologically or pathologically abnormal milks may be detected by the resazurin test as compared to the methylene blue test. By using the resazurin test a greater saving of time is effected. H.P.

60. **Thermal Death Range of Bacteria in Milk.** F. W. GILCREAS AND J. E. O'BRIEN, Research Laboratories, New York State Department of Health, Albany, N. Y. Jour. Milk Technol., *9*, 5 : 269–272. Sept. and Oct., 1946.

See Abs. 21, Jour. Dairy Sci., *30*, 2 : A14. Feb., 1947.

CHEESE

61. **Factors Influencing Acid Production by Cheese Cultures. I. Effect of Cooking Temperatures on Acid Production in the Manufacture of Cheddar Cheese.** F. J. BABEL, Iowa Agr. Expt. Sta., Ames. Natl. Butter and Cheese Jour., *38*, 1 : 34. Jan., 1947.

An abbreviated form of the original publication in Jour. Dairy Sci., *29*, 9 : 589. Sept., 1946. W.V.P.

62. **Making Process Cheese in Small Plants.** C. R. BARKER, Oak Park, Ill. Natl. Butter and Cheese Jour., *37*, 12 : 90. Dec., 1946.

Cheese that has been aged for 30 days at 60° F. without paraffin can be used to make a satisfactory processed cheese. Water lost in curing is replaced during processing. In blending, there should be 50% of cheese

"on the sweet side" and 50% "on the acid side". Cooking this blend of cheese with water in the jacket of the cooker at a pressure of 10 lbs. and a temperature of 239° F. is recommended. (No warning is given concerning ability of the cooker to stand the pressure.) W.V.P.

63. Practical Suggestions on the Manufacture of Process Cheese. C. R. BARKER, Oak Park, Ill. Natl. Butter and Cheese Jour., *38*, 1 : 42. Jan., 1947.

Cheese made in a single factory can be processed successfully if some is made "on the acid side" and some "on the sweet side". Vat drippings of 0.9% before salting give cheese of the acid type. Such cheese should constitute 30–40% of each batch, while the remainder should be "sweet" from curd with vat drippings of 0.6% at salting. Tough, over-cooked Cheddar is not desirable. The usual cleaning and cooking operations are described very briefly. A diagram illustrates the flow of material through the operations of curing, grading, blending, cleaning, grinding, cooking, packaging, cooling, and shipping. W.V.P.

CHEMISTRY

64. Some Chemical Changes Produced in Milk by High Temperature Heat Treatment. IRA A. GOULD, JR., Dairy Dept., University of Maryland. Milk Plant Monthly, *35*, 9 : 70–71. Sept., 1946.

The author reviews brifly results of his research in this field during the past 12 years, pointing out the relationships between the boiled or cooked flavor of milk and some chemical changes bringing out these flavors. The cooked flavor was caused by the formation of sulfhydryl compounds, usually accompanied by the evolution of hydrogen sulfide gas at temperatures of 168.8–172.4° F. These chemical changes become more pronounced as the temperature increases to boiling. Serum proteins were undoubtedly the principal contributors to the formation of the heat-labile sulfides. Extremely small quantities of metallic salts of mercury, silver, ferric iron, and copper influence sulfide liberation. Additions of small percentages of sucrose, glucose, and lactose lower sulfide liberation. On the other hand, cysteine hydrochloride, sodium cyanide, sodium sulfite, and ethyl alcohol favor sulfide liberation. Prolonged heating of milk at high temperatures produced further changes in sulfide liberation, which is associated with a caramel flavor and a brown color. High heat treatment, particularly under pressure, produced appreciable increases in titratable acidity and destruction of a considerable portion of the lactose. This destruction is favored by the presence of sodium citrate or disodium phosphate. Salts of milk play a significant rôle in the amount of titratable acidity increase. Less than 10% of the acidity was due to lactic acid and 50 to 60% of the heat-

produced acidity was found to be formic acid. This work shows the complexity of the changes occurring in milk at high temperature under various conditions.   G.M.T.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

65. The Future of Dry Milk. Stephen O'Dea, U. S. Dept. of Agr. Natl. Butter and Cheese Jour., *38*, 1: 80. Jan., 1947.

Increased use of dry milk in some areas through the school lunch program would improve the health of children. Government-owned facilities for producing dehydrated dairy products are located in five states. "It is not feasible to close down the drying facilities and shut off these outlets for farmers' whole milk." Some expansion in U. S. production of nonfat milk solids seems likely. "More thinking needs to be done about the development of markets for nonfat dry milk than for any other manufactured dairy product."   W.V.P.

66. The Manufacture and Use of Condensed Cheese Whey and Crude Whey Protein. B. H. Webb and C. F. Hufnagel, U. S. Dept. of Agr., Washington, D. C. Natl. Butter and Cheese Jour., *37*, 12: 34. Dec., 1946.

Cheese whey can be condensed to 1/10th its volume for 1 to 1.8¢ per lb. of finished product. For human food the whey is pasteurized as soon as it is removed from the cheese. It can be condensed with or without sugar. Unsweetened whey is concentrated to 65–70% total solids. Crystallization of the lactose is controlled by prompt condensing in a clean pan, drawing concentrate as a clear sirup, cooling rapidly at 90° F., seeding with lactose crystals, and stirring. Plain condensed whey with a pH of 4.5 or less can be packed in air-tight barrels and kept for several months at cool temperatures. Sweetened condensed whey (see Jour. Dairy Sci., *21*: 305–314, 1938.) is made by separating, pasteurizing, adding sugar in amounts to equal the weight of whey solids, and condensing to 76% total solids. This concentration at 122° F. gives 1.360 sp. gr. (38.4° Be). The concentrate is cooled to 95° F., seeded, stirred slowly for 1 to 3 hr., and packed in barrels or cans. Refrigerated storage is not required. Whey protein for pharmaceutical uses is separated from whey by heat and acid. The curd is washed with water, drained, pressed, and preserved by drying or freezing. Albumin curd can be dried rapidly at 110–120° F. in a tunnel drier. Some pharmaceutical companies will furnish specifications of purity for albumin powder. The residue can be concentrated for feed or milk sugar. Equipment for sugar manufacture can be supported only by large operations.

Successful commercial uses for condensed whey in food products are

limited at present to confections, bakery goods, and cheese foods. Characteristic flavor and the insolubility of its lactose make whey inferior to skim milk for food purposes. It has high nutritive value and low cost. Formulas are given for whey candy and cookies. Methods of using whey products in bread, sweet baked goods, cheese foods and cream soups are suggested.

W.V.P.

67. A Method for Producing a Dairy Spread. K. G. WECKEL, Dept. of Dairy Industry, University of Wisconsin, Madison, Wis. Milk Plant Monthly, *35*, 9: 24–25. Sept., 1946.

A method for producing a dairy spread containing 28% butterfat, 19% milk solids-not-fat, with and without added vitamins, is described and illustrated. The ingredients are whole milk powder, cream, buttermilk, lactic acid, salt, vitamins A and D, and starter distillate. After pasteurizing, the spread is homogenized at a pressure which will give the greatest plasticity without graininess (usually varying from 1,500 to 2,500 lbs.). The product is packaged hot, preferably in glass containers of the cottage-cheese jar type. The product sets upon cooling and may be kept 1 or 2 weeks, similar to any soured milk product.

G.M.T.

68. Milk Sugar. GERTRUDE G. FOELSCH AND HARRY C. TRELOGAN, Production and Marketing Administration, Washington, D. C. Milk Plant Monthly, *35*, 11: 40–41, 48. Nov., 1946.

Renewed interest is being manifest in milk sugar because it is playing an important rôle in penicillin production. A program was inaugurated in 1943 to increase milk sugar production from cheese whey. Manufacturers find partial recovery of crude milk sugar more profitable, using the remaining mother liquor to produce poultry feed. Three grades of commercial milk sugar—crude, technical, and refined—are produced. Emphasis today is being placed on production of the crude form for use in the manufacture of penicillin, although formerly milk sugar was consumed largely in infant foods.

G.M.T.

FEEDS AND FEEDING

69. Vitamin A Requirements in Calves, Part I. J. M. LEWIS AND L. T. WILSON. New York University College of Medicine, New York City, and The Walker Gordon Laboratories, Plainsboro, N. J. Cert. Milk, *21*, 244: 5. Aug., 1946; Part II, Cert. Milk, *21*, 245: 9. Sept. and Oct., 1946.

Six groups of four calves each were fed various levels of vitamin A, ranging from 32 to 1,024 USP units per kg. of body weight per day. Data were obtained on rate of growth, blood levels of vitamin A, and liver stor-

age. Results indicate that 32 units per kg. of body weight apparently satisfies the minimum requirements. Maximum growth was obtained on an intake of 64 USP units per kg. of body weight. The concentration of vitamin A in the blood was proportional to the intake until 512 units were given, at which level maximal blood concentrations were obtained. In general, liver stores were quite low for calves receiving 32, 64, and 128 units per kg.; moderate amounts of vitamin A were found in the livers of those fed 256 to 512 units, and larger amounts in the group fed 1,024 units. From the standpoint of both growth and liver storage, the daily intake of vitamin A for young calves should be about 250 units per kg. of body weight or 11,000 units per 100 lbs. of liveweight. Thus, the vitamin A requirements in calves are of the same order of magnitude as in young rats and in infants.

W.S.M.

FOOD VALUE OF DAIRY PRODUCTS

70. **Between Meal Milk Drinks Beneficial for Children.** National Dairy Council. Canad. Dairy and Ice Cream Jour., *25*, 11: 78. Nov., 1946.

No adverse effect on the appetite or well being of children ranging in age from 3 to 14 years when fed milk 1 hr. before meals was observed. Tests on 59 children revealed an average stomach-emptying time of 118 min., representing a range of 50 to 170 min. The contributions of two levels of milk (44 and 63 oz.) to the total daily nutrient intake were, respectively: 40 and 49% for calories, 35 and 45% for protein, 85 and 91% for calcium, 30 and 41% for vitamin A, 55 and 65% for thiamine, and 80 and 87% for riboflavin. Each 7 oz. serving of milk contributed approximately 5% of the total caloric intake. H.P.

ICE CREAM

71. **Good Methods of Manufacture for Dry Ice Cream Mix.** S. T. COULTER. Canad. Dairy and Ice Cream Jour., *25*, 11: 61. Nov., 1946.

See Abs. 42, Jour. Dairy Sci., *30*, 2: A20. Feb., 1947.

MILK

72. **Quality Milk from Cow to Milk Plant.** C. B. A. BRYANT, Johnson & Johnson, Chicago, Ill. Milk Plant Monthly, *35*, 12: 26–27, 52–53. Dec., 1946.

Emphasis is placed upon keeping sediment out of milk, as sediment is one of the common causes of milk rejections. Despite care in cleanliness

during the production of milk, sediment gets into milk from loosely covered cans or from nonrinsed cans prior to use. Wind-blown dust is often the cause of sediment in milk. Lids of cans, as well as the can itself, should be protected from dust. Rinse water on the farm may be the source of sediment. Displaying the sediment disc and pointing out the common causes for sediment in milk are aids in keeping the milk clean. The responsibility for clean milk rests not only with the producer but also with the hauler and processor.   G.M.T.

73. **Evaluation of Factors when Processing Homogenized Milk.** E. M. GIBERSON. Canad. Dairy and Ice Cream Jour., *25*, 11 : 52. Nov., 1946.

Homogenized milk always must be pasteurized, clarified, and processed rapidly and at proper temperatures and pressures. The advantages of homogenized milk are that it produces uniformity of appearance, pouring characteristics, flavor, and color. Before a plant operator purchases equipment and starts processing homogenized milk, he should consider type of pasteurization to be employed, clarification and filtration methods, capacity of various pieces of equipment, and advantages and disadvantages of each. The equipment needed for homogenizing milk requires a considerable capital investment in milk processing equipment.

74. **Flavors in Milk Influenced by Pastures and Cattle Feeds.** JACK BAILEY. Canad. Dairy and Ice Cream Jour., *25*, 11: 59. Nov., 1946.

The problem of preventing the flavors of feed from getting into the milk depends on the time of feeding. It is generally agreed that feed flavor is no longer evident in the milk 5 hr. after feeding. The cows should be kept in an atmosphere free from undesirable odors before milking. If the flavor is due to silage or barn feeds, it is advisable to feed after milking. If the flavor is due to pasture weeds or feeds, it would seem best to bring the cows off the offending pastures preferably 5 hr. and at least 2 hr. before milking. The development of a cowy, old, stale, and then rancid flavor in milk is due to lipase action. This activity is aggravated by shaking whole warm raw milk and by milking cows in advanced stages of lactation. The mixing of milk likely to become rancid with four or more parts of normal milk always will prevent rancidity. In pasteurized milk, oxidized flavor may be caused by absence of bacteria. Metallic, fishy, oily, and tallowy flavors, which are common in pasteurized milk, are caused by dissolved oxygen, copper contamination, oxidase enzyme, and exposure of milk to direct sunlight.
                                                              H.P.

75. Control of Milk Watering. PAUL CORASH, Dept. of Health, New York, N. Y. Milk Plant Monthly, *35*, 10: 90, 92–93, 96. Oct., 1946.

A study of the detection of milk watering by means of the Hortvet cryoscope, an instrument essentially adaptable to laboratory use but chosen for field use, indicated a relatively large percentage of the samples of producers' milk examined had been watered. The lactometer was used to screen out suspicious cases. Calculating the solids-not-fat content of milk furnished the basis also for judging whether or not a sample of milk should be tested with the cryoscope. Importance is placed on securing relatively fresh samples in order to secure correct cryoscope values. Other methods, such as the copper-serum method and the chemical determination of fat, total solids, and ash on deck samples, also may be used in determining watering, but are considered less accurate than the cryoscope method.

G.M.T.

MISCELLANEOUS

76. Vacreation of Cream, Milk, and Ice Cream Mix and Condensing Milk with the Vacreator. G. H. WILSTER, Oregon State College, Corvallis, Ore. Milk Plant Monthly, *35*, 11: 28–32. Nov., 1946.

The vacreator is illustrated and the steps involved in its operation are described fully, the process consisting briefly in heating the milk product to approximately 200° F. and passing it through a series of chambers with increasing vacua until the product is removed at a markedly lower temperature. The process removes gases and off-odors present in the product; consequently the finished product will be free of off-odors. Butter manufactured from cream by the evacuation process had a higher score than that not treated. Ice cream of an excellent quality was produced from vacreated ice cream mix with which vacreated condensed milk was used.

G.M.T.

77. Recent Developments in Dairy Manufacturing Through Research. G. H. WILSTER. Canad. Dairy and Ice Cream Jour., *25*, 9: 34; 10: 54. Sept. and Oct., 1946.

Some of the recent developments in dairy manufacturing based on research are: (1) the continuous high-temperature short-time pasteurization of milk in a totally enclosed apparatus using clarification, homogenization, and an enclosed cooler; (2) single service containers for milk; (3) sterilized cream; (4) sterilized milk; (5) the use of the vacreator in the dairy industry; (6) metal churn for buttermaking; (7) dry butterfat or butteroil; (8) continuous butter churns; (9) curing cheese in valve-vented cans; (10) packaging rindless cheese in moisture-proof sheets; (11) Army cheese

spread; (12) dry ice cream mix; (13) dry whole milk; (14) dry cream mix for whipping by aeration; (15) sweetened dry nonfat milk solids; (16) frozen concentrated milk; (17) use of cheese whey for candy, soups, puddings, plastics, penicillin; and (18) dairy spreads.   H.P.

78. **Flocculation and Sterilization Without the Use of Chemicals.** W. R. MARSHALL. Canad. Dairy and Ice Cream Jour., *25*, 10 : 27.   Oct., 1946.

Water can be purified and sterilized in any flow-rate gravity or pressure system by the use of aluminum electrodes as a flocculator and silver as a sterilizer. The flocculating unit operates on a D.C. current of 6 volts, 3 amperes, which supplies the current for the flocculating electrodes. Unlike ordinary sand filters which depend on chemicals alone to produce the floc, the system is not affected by the lower temperature of the water. Immediate sterilization is obtained since the coagulant produced by the aluminum electrode holds the silver in an ionized form, immediately attracting and destroying bacteria. The amount of silver ions needed to sterilize water is in the neighborhood of one part in ten million. With this apparatus, which consists of a sand filter tank with rapid flocculators to remove the dirt and the silver electrode to destroy the bacteria, use of any chemicals is not necessary.   H.P.

79. **The Use and Abuse of Wetting Agents as Applied to the Cleaning of Milking Machines.** HARLOW L. PENDLETON, Massachusetts Department of Agriculture. Milk Plant Monthly, *35*, 12 : 30–32, 70, 72.   Dec., 1946.

Experimental data indicated that the flush washing of milking machines using wetting agents was as effective in reducing bacteria counts of milking machine units as brush washing. Also, the cold water prerinsing of milking machines could be eliminated for all practical purposes. After 7 days' treatment, low counts were obtained on milking machines, with no appreciable increase after 14 days. Milkstone deposits were insignificant at the end of 7 days. The flush-washing method of cleaning milking machines, employing wetting agents, was not advocated as a cure-all for cleaning. The process should be used with caution by the careless milk producer. However, the careful operator can clean the machine units with less time and labor when using this method than by brush washing. Until more is known of various detergents and cleaning qualities of wetting agent compounds, intermittent use of brush washing of milking machines must be recommended. Carefully selected detergents with wetting properties, intelligently used, should prove a great boon to the dairy farmer.

G.M.T.

80. **Labor-saving Methods and Materials for Dairy Plant Cleaning.**
D. H. JACOBSEN, Cherry-Burrell Corporation, Chicago, Ill. Milk
Plant Monthly, *35*, 11: 24–27, 36. Nov., 1946.

Advancements in machinery design and building materials and layouts
make imperative higher standards of plant sanitation despite higher labor
costs. Choice of products for cleaning in milk processing plants often is
made on bases of wetting properties, water-softening powers, costs, and
availability. Properties desired in a good cleaner are: (1) quick and com-
plete solubility, (2) non-corrosive on metal surfaces, (3) complete water-
softening or water-conditioning power, (4) good wetting or penetrating
action, (5) emulsifying action on fat, (6) dissolving action on milk solids,
(7) deflocculating, dispersing, or suspending action, (8) good rinsing prop-
erties, (9) germicidal action, (10) economy in use. Straight alkalies, acids,
or wetting agents alone do not meet the requirements of a good cleaner.
Likewise, a universal cleaner does not exist, since it is not practical to use
one cleaning agent on all jobs. Hardness of water plays an important rôle
in the efficiency of the cleaner. Phosphates improve the action of all dairy
cleaners in hard water. Wetting agents are generally the most expensive
component of dairy cleaners. Proper lighting, adequate ventilation, use of
pipe wash tanks, storage racks, plant layouts, and machines influence the
speed and effectiveness of cleaning. Circulating cleaning solutions and
spray systems offer promise in reducing hand labor in dairy plants. Both
acid- and alkaline-type circulating cleaning solutions are used in enclosed
systems, such as plate heat exchange systems. Spray systems have decided
advantage in dairy plant cleaning for large tanks or vats and surface cool-
ers. Portable cleaning units offer a possibility in facilitating cleaning
operations. G.M.T.

81. **Cleaning Electrical Windings.** D. L. GIBSON, Westinghouse Electric
& Manufacturing Co., East Pittsburgh, Pa. Natl. Butter and
Cheese Jour., *38*, 1: 40. Jan., 1947.

Methods of cleaning motors are varied to suit the type of cleaning job
required, *i.e.*, removal of grease, softening of varnish for rewinding, re-
moval of effects of exposure to chemicals or flood waters. Standard methods
of cleaning include rubbing with lintless cloths soaked in selected petroleum
distillates or carbon tetrachloride, use of vacuum cleaning apparatus, spray-
ing or immersing in solvents, and washing with water. It is not advocated
that facilities be kept available for such diverse methods, but rather that the
job of cleaning be analyzed carefully to save money, time, and labor.

W.V.P.

82. Determination of Refrigerant Pipe Size. H. M. HENDRICKSON, Preston Construction Co., Division of Safeway Stores, Oakland, Calif. Refrig. Engin., *52*, 4: 317–325. Oct., 1946.

The author emphasizes that pressure drop, and not velocity, is normally the governing factor to be employed in sizing refrigerant piping. The chief consideration of refrigerant velocities is to keep them low enough to eliminate excessive noise in the lines, and not so low as to interfere with proper oil return. The author presents tables and charts for the more common refrigerants, with the greatest emphasis on the two most popular (Freon 12 and ammonia) giving the best available information on pressure drop to facilitate the determination of the proper size of refrigerant mains.

L.M.D.

83. Motor Transport Refrigeration. Part I—A Modern Refrigerating Unit. HENRY O. KIRKPATRICK, Advance Manufacturing, Inc., Detroit, Mich. Refrig. Engin., *52*, 6: 521–524. Dec., 1946.

The modern trend in truck refrigeration is toward the self-contained or package-type unit, comprising a compact installation of gasoline engine with starter, compressor, condenser, and receiver fastened on a rigid frame. Above the frame is an insulated platfrom upon which is located the evaporator, heat exchanger, expansion valve, and oscillating fan. The lower portion is closed from the truck body with an insulated bulkhead. Access to the lower compartment of the unit is had by two doors in the front wall of the trunk through which minor service can be rendered or control valves operated. Major servicing can be done from the interior by removal of the bulkhead. Replacement is readily made with a new unit if shop tear-down is required. In this modern unit provision is made to use the reverse cycle employing the refrigerating unit as a heat pump, the evaporator functioning as a condenser and the condenser as the evaporator. This versatility enables the operator to maintain a truck temperature of 0° F. against 80° F. outside or to maintain a temperature of 68° F. inside against that of −15° F. outside if protection of that sort is needed. The frame installation of the mechanical components of the unit overcomes the road vibration encountered in truck transportation. To protect against excess pressures during shut down, hand valves (six in case of the reverse cycle unit) all are closed completely, isolating the compressor from the rest of the system. Automatic regulation of suction pressure to 30 psi prevents overload during pull-down of truck body temperature from 100° F. to 35° F. design temperature. Another trend will be that of units of 3 to 5 ton refrigeration capacity, capable of maintaining −10° F. with 3 in. insulation, instead of the present 1 to 2 ton units used to maintain 32° F. with 3 in. insulation or 10° F. in trucks insulated with 6 in. of insulation. This is because the larger capacity

unit takes up little more loading space and together with 3 in. insulation weighs much less than the smaller unit with 6 in. insulation. Also, there is a much greater gain in pay load space, resulting in a lower operating cost per ton hauled. L.M.D.

84. The Use of Silica Aerogel as a Thermal Insulation. F. FAXON OGDEN AND JOHN F. WHITE, Monsanto Chemical Co., Merrimac Division. Refrig. Engin., *53*, 5 : 411–414. Nov., 1946.

Silica aerogel is a light, free flowing, voluminous solid having a density of about 7.0 lbs. per cu. ft., approximately 94% of its volume being air. It has a k factor about 10% less than the theoretical value for still air. This is explained on the basis of the pore diameter in the aerogel being about 250 Ångstroms, which is less than the mean free path of the molecules in free air. This causes a reduction in the molecular movement of the air enclosed in the pore spaces and lowers the conductivity of that air below normal. For a mean temperature of 0° F., k is given as 0.13 and for −50° F., as 0.115. Moisture-vapor imperviousness is practically 100% for silica aerogel, but it must be protected against liquid water, for when over 15% by weight is absorbed, the aerogel structure collapses and cannot be restored to its original state. Equilibrium in natural settling is reached after about 5 hr. Mechanical vibration will hasten settling, and by this means speed up in filling cabinet spaces may be obtained. When silica aerogel is subjected to mechanical load, there is initially a relatively large decrease in volume due to closer packing of the individual particles. This is not recovered upon release of pressure. Fire hazard is nonexistent because the material for insulation is heat-treated to remove 7 to 10% volatile matter of inflammable nature. This material is not silicotic, but a respirator mask is advised because of the dust. Because its k factor is about one-half that of materials used for freezer cabinet insulation, a great increase in volume of storage space may be realized without increasing external dimensions, while in the ordinary refrigerator an increase in capacity of between 80 and 90% may be realized. When used in normal spaces designed for other insulating materials, the low conductivity of silica aerogel reduces refrigeration unit operation 40 to 50%. Research is being continued and has already resulted in a product of 3.5 to 4.0 lbs. per cu. ft., retaining all the favorable properties of the original aerogel. L.M.D.

85. Swedish Insulant Offers Useful Properties. THORE M. ELFVING, Stockholm, Sweden. Refrig. Engin., *52*, 4 : 311–313. Oct., 1946.

Details are given of the physical properties of Isoflex, a thermal insulant of air layer type made from thin corrugated foils of cellulose acetate. The foils have a thickness of approximately 0.0015 in. These thin foils are

joined with their corrugations at right angles, making a punctiform contact between them. The several foils are joined into slabs of varying thicknesses without adhesive material, being "welded" together by fusing the cellulose acetate at the points of contact. The slabs are 24 in. by 24 in. In order to impart black body property to the cellulose acetate foil, which is much thinner than 0.004 in., an opacifier is added to the cellulose acetate before forming into the foil sheets. This insulant is very light, weighing only 0.67 to 0.8 lb. per cu. ft. It can be cut readily to fit into irregular spaces.

In applying Isoflex, the only requirement is that the slabs fit tightly. The insulating layer should be slightly thicker than the space allowed for it, so that the slabs are compressed slightly. Its flexibility lends it admirably to the insulation of tank trucks for transportation of vegetable oils, milk, and other goods. The thermal conductivity of Isoflex is not given.

L.M.D.

ABSTRACTS OF LITERATURE

BOOK REVIEWS

86. Milk Marketing under Federal Control. CARL McFARLAND, Formerly Assistant Attorney General of the United States. 205 pages. $7.50. Milk Indus. Found., Washington, D. C. 1946.

This book deals in a technical legal manner mainly with regulation conducted under the Agricultural Marketing Agreement Act of 1937. The growth in the last 50 years of legislation by administrative directive rather than by statute is commented upon. Most dairy laws in the past have consisted of rather narrow and specific governing statutes with provision for judicial enforcement. Broad administrative powers are given in the Food, Drug and Cosmetic Act of 1938 and still broader ones in the Agricultural Marketing Agreement Act of 1937. The official code carries the last four sections of the 1937 act under the chapter title ''Agricultural Marketing Agreement Act'' and the remainder under the ''Agricultural Adjustment Act of 1933''.

The essential substance of the Marketing Act is the fixing of minimum producer prices to be paid and prescribing how they are to be paid by handlers. In discussing detailed procedure and administration, the statement is made that the Marketing Act prescribes what is in some essential respects the most complicated procedural system on the Federal statute books. Detailed steps are outlined covering mediation and arbitration, the marketing agreement and order procedure, administrative and judicial review of orders, enforcement provisions and methods, and, finally, organization and personnel. Responsibility for the administration of the act rests with the Secretary of Agriculture. The final chapter gives a summary of typical marketing order provisions and a review of the issues which underlie them.

Appendices include a transcript of the Act, rules of practice and procedure, and marketing order references to the Federal Register for 49 different markets. E.F.G.

87. Bovine Mastitis. A symposium edited by RALPH B. LITTLE AND WAYNE N. PLASTRIDGE. 546 pages, illustrated. $7.00. 1946. McGraw-Hill Company, Inc., 330 West 42nd St., New York 18, N. Y.

This book is a thorough and critical treatment of the various aspects of this widespread and costly disease of dairy cattle. The editors and publisher are to be commended for their method of having the various sections or chapters written by recognized authorities in the field. The subject is covered in a broad and critical sense and wide differences of opinion recog-

nized. The anatomy of the udder, the physiology of milk secretion, the pathology, diagnosis, bacteriological classification, transmission, treatment, control and public health significance of mastitis are among the subjects covered. An appendix giving details of tests, laboratory techniques, and methods is a useful addendum. Although this book is prepared in the form of a symposium, it should prove to be a useful text for students and investigators in the field and a reference work for veterinarians, dairymen, public health officials, and milk sanitarians.   T.S.S.

88. **The Problem of Fertility.** Edited by EARL T. ENGLE. 254 pages. $3.75. 1947. Princeton University Press, Princeton, N. J.

This publication consists of a series of 16 papers presented at the Conference on Fertility sponsored by the National Committee on Maternal Health. These papers cover fields of active interest and investigation relating to the problem of fertility in man and animals. The inclusion of the discussions which followed the presentation of each paper provides additional interesting information and points of view.

The following subjects are presented: Patterns of Estrous Cycles, by S. A. Asdell; Ovulation in Sheep and Goats, by R. W. Phillips, R. M. Fraps, and A. H. Frank; Induction of Ovulation and Subsequent Fertility in Domestic Animals, by L. E. Casida; the Induction of Ovulation in Domestic Animals, by John Hammond; the Ovary at the Time of Ovulation, by George W. Corner; Hormonal Control of Ovulation, by H. H. Cole; Cervical Mucus and the Menstrual Cycle, by W. T. Pommerenke and Ellenmae Viergiver; Spermatozoa and Cervical Mucus, by A. R. Abarbanel; Glycolosis, Livability, and Fertility of Bovine Spermatozoa, by G. W. Salisbury; Metabolism and Motility of Human Spermatozoa, by John Macleod; Fertilizing Capacity of Rabbit Spermatozoa, by M. C. Chang; Biology of Equine Spermatozoa, by Victor R. Berliner; Artificial Insemination of Dairy Cattle, by J. W. Bartlett; The Cervix Uteri in Sterile Matings, by Fred A. Simmons; The Effect of Synthetic Thyroprotein on Sterility in Bulls, by E. P. Reineke; and Methods of Determining the Time of Ovulation in Domestic Animals, by John Hammond.

This book should prove of special interest and value to those engaged in the various phases of artificial insemination work. The discussions are well supported by experimental data and illustrations.   T.S.S.

BACTERIOLOGY

89. **The Growth of Coliform Bacteria in Pasteurized Milk Stored at Refrigerated Temperatures.** A. C. DAHLBERG, Dept. of Dairy Ind., Cornell University, Ithaca, N. Y. Dairy Indus. Found. Assoc. Bull., *39*, 4: 86–95. Jan. 10, 1947.

Results previously published by the author (Jour. Dairy Sci., *29*: 651–

655, 1946) are given in detailed tables which show that coliform bacteria increase more rapidly in numbers than other bacteria in pasteurized milk held at refrigeration temperatures. Coliform bacteria were present in the majority of freshly pasteurized milk in one quart volumes. After storage at 45–50° F. and 55–60° F., the coliform bacteria constituted significant percentages of the total bacterial content. Since coliform bacteria in themselves are not a public health problem unless present in excessive numbers, a standard for condemnation might well be one that can be met by the best sanitary plants in the summer when counts are naturally the highest. No numerical standard is proposed by the author. E.F.G.

90. **Coliform Bacteria Problem and Its Control.** A. C. DAHLBERG, Dept. of Dairy Ind., Cornell University, Ithaca, N. Y. Milk Dealer, *36*, 4 : 130–133. Jan., 1947.

See preceding abstract.

91. **Coliform Organisms in Pasteurized Milk.** C. J. BABCOCK, Market Milk Specialist, B.A.I., Agr. Res. Admin., U. S. Dept. Agr., Washington, D. C. Milk Indus. Found. Assoc. Bul., *39*, 4 : 78–85. Jan. 10, 1947.

During the war the Medical Department of the Army made extensive application of the coliform test at Army installations throughout the country as an index to the sanitary conditions under which pasteurized milk is handled. Milk to be tested was inoculated into five tubes. If three or more of the tubes showed fermentation, the sample was considered positive for coliform organisms. Army installations adopting this method obtained very satisfactory results. A positive test may be due to improper pasteurization, heat-resistant organisms, excessive contamination of raw milk, or growth after pasteurization. There was no correlation between the results of the coliform test and the standard plate count. Positive and negative coliform results were obtained on both low and high count milk. Whenever coliform organisms are found in the milk delivered to the consumer, the condition almost invariably can be corrected by a thorough cleanup of the plant equipment. Thus the test is an ideal means of checking the cleanup operations in a plant. E.F.G.

92. **The Viability of Dried Skim-Milk Cultures of *Lactobacillus bulgaricus* as Affected by the Temperature of Reconstitution.** MARVIN L. SPECK AND ROBERT P. MYERS, National Dairy Products Corp., Baltimore, Md. Jour. Bact., *52* : 657. Dec., 1946.

"In spray-dried skim-milk cultures of *Lactobacillus bulgaricus* a large number of cells that failed to grow when the temperature of the reconstitu-

ting fluid was 21 to 25° C. were activated sufficiently to produce normal growth when the temperature of the reconstituting fluid was 37 to 50° C. When the culture was reconstituted at 21 to 25° C. and warmed to 50° C., the activating effect of the heat was not obtained.

"The increase in the colony count resulting from reconstitution at 50° C. over reconstitution at 21 to 25° C. could not be explained by an increase in the solubility of the powder, nor an increase in the dispersion of the cells.

"Reconstitution of dried *L. bulgaricus* at 50° C. with subsequent inoculation into skim milk showed greater activity in the skim milk, particularly in the early stages of growth, than was obtained when the culture was reconstituted at 21 to 25° C.

"Freeze-drying part of a skim-milk culture that was also spray-dried showed that cells in the freeze-dried culture were not only activated by reconstitution at 50° but that this temperature actually was lethal to many of the cells. This suggested a physiological difference between freeze-dried and spray-dried cells of *L. bulgaricus,* since the latter were markedly activated by heat."   D.P.G.

93. **Spotting and Evaluating Biological Dirt.** M. C. JAMIESON, H. J. FORSTER, AND A. REY. Canad. Dairy and Ice Cream Jour., *26*, 1: 28. Jan., 1947.

Biological dirt ordinarily is invisible dirt that contains bacteria, spoils food products, and sometimes causes sickness and even death. The suitcase type laboratory developed for assisting eating and drinking establishments in sanitation problems (Abs. no. 446, Jour. Dairy Sci., *29*, 12: A206, Dec., 1946) has been extended to the dairy industry. "Jamieson's Sanitation Kit" has been used by a few dairies and by the Manitoba Department of Public Health. The test is simple and requires little technical training or scientific interpretation. The representative area of surface is swabbed off; the swab is smeared over the medium prepared in convenient, small, screw-capped bottles and incubated at room temperature for 3 days. The fewer the colonies that develop, the cleaner the equipment. All plant employees see the results and take an active interest in producing clean culture bottles. Application of this test for farm use is promising.   H.P.

94. **Vitamin A and Carotene Content of Ontario Butter.** W. H. SPROULE, F. W. HAMILTON, C. E. LACKNER, S. H. JACKSON, T. G. H. DRAKE, AND M. MOFFAT. Canad. Dairy and Ice Cream Jour., *25*, 12: 23. Dec., 1946.

The mean vitamin A potency of butter from 21 creameries representing the five geographical regions of Ontario was 13,269 I.U. per pound. The monthly mean for the period May to November was 14,702 I.U. and from

December to April 9,915 I.U. per pound. During the periods mentioned, carotene supplied 28.8, 25.2, and 17.6% of the total vitamin A potency of Ontario butter. The vitamin A and carotene contents of butter vary with the month of the year as well as with different regions of production, depending primarily upon pasture supplies. The peak values for both vitamin A and carotene were reached in the month of June, followed by a downward trend during the warm midsummer and early autumn period. The latter value returned to almost the June level in October. A slight decline took place in December, which continued throughout the winter, when the lowest values for the year were recorded.　　　　　　　　　　　　　　H.P.

CHEESE

95. A Study of Canadian Cheese on United Kingdom Markets. WM. C. CAMERON. Canad. Dairy and Ice Cream Jour., *26*, 1: 33. Jan., 1947.

There is a market in Great Britain for select cheese of uniformly high quality, for which a premium will be paid. To reach this objective, the three main points which warrant special care and attention are: (1) clean milk supply, (2) amount of acid in the finished cheese, and (3) closeness of body. Excellent cheese is one having a close-boring, meaty texture with a clean, nutty, cheddar flavor. Two major defects found in Canadian cheese were openness and curdy body, the latter varying from a dry texture to a chalky or pasty texture. It was generally agreed that in most cases where openness was found the curd had not been sufficiently matured on the pan before milling and later, before salting. It is claimed that too much "added acid" in the form of starter is detrimental to the Cheddar cheese. Not over 1.5% starter is recommended if the cheese is not to become acidy on aging.　　　　　　　　　　　　　　　　　　　　　　　　　　H.P.

96. Give the Cheese of the Lower Fat Type the Place Due It—20% Steppe Cheese. F. L. (FRANK LAMBERTSEN), Nordisk Mejeri-Tidsskrift, *12*, 6: 109–110. 1946.

Cheese made from partly skimmed milk should be produced more than it actually is. This cheese always will have a market because it is cheaper than cheese made from whole milk and it contains the same amount of the nutritionally important solids, protein, and calcium salts.

Steppe-cheese is a square-formed cheese, weighing from 11–13 lbs., with an elastic consistency. Cut, it shows evenly distributed eyes about $\frac{1}{4}$ in. in diameter. A method that has been used for making this cheese for many years in a Danish factory is given. The milk used is pasteurized. Before the renneting, 2–3% starter, 5% water, and calcium chloride (if necessary) are added to the milk. Rennet at the rate of 230 cc. per 1,000 lbs. of milk

(50 cc. per 100 kgs.) is added at 97° F. and the setting period is 40 min. The curd is cut by 1-cm. knives and stirred for 20 min., after which a little more than half of the whey is drained off. Now the curd is stirred vigorously by a fork and heated quickly to 106° F. by adding boiling water, while the vigorous stirring is continued. Some more whey is drained off and the stirring of the curd finished within about 40 min. Salt, 2.5 lbs. per 1,000 lbs. of milk (250 g. per 100 kgs.), is added to the water used for heating. The stirring finished, the curd is cooled down to 99° F., drawn to the end of the vat, and piled for about 20 min., after which it is cut in blocks, hooped and pressed in a cheese press for 45 min. After pressing the cheese is placed in cold water over night and next morning in brine for 72 hr. After a short period in a cooler at 61° F., the cheese is finished at 54° F. and 80% humidity. The cheese can be stored for 7–8 months.   T.K.

CHEMISTRY

97. Colorimetric Determination of DDT in Milk and Fatty Materials. M. S. Schechter, M. A. Pogorelskin, and H. L. Haller, U. S. Dept. Agr., Agr. Res. Admin., Bur. of Entomology and Plant Quarantine, Beltsville, Md. Analyt. Chem., *19*, 1: 51–53. Jan., 1947.

Recent work has shown that contamination of milk, butter, eggs and meat may result when farm animals consume DDT-treated feed; that ingested DDT accumulates as such in the fatty tissues of experimental animals and can be excreted in milk; and that such milk may become toxic enough to kill other animals drinking it. This report describes a procedure for the determination of DDT as such in foodstuffs containing considerable amounts of fatty matter. The method is not rapid but it permits the detection and determination of DDT in milk in quantities as low as 1 p.p.m. Milk is extracted with Skellysolve B, the emulsions are broken by means of a centrifuge, and DDT is separated from the fatty fraction by a sulfuric acid treatment based on the solubility of fats and the insolubility of DDT in concentrated sulfuric acid. The DDT residue is nitrated and DDT is determined by spectrophotometric measurements.   B.H.W.

98. Estimation of DDT in Milk by Determination of Organic Chlorine. R. H. Carter, U. S. Dept. Agr., Agr. Res. Admin., Bur. of Entomology and Plant Quarantine, Beltsville, Md. Analyt. Chem., *19*, 1: 54. Jan., 1947.

The method presented describes a procedure for estimation of DDT in milk and butter samples by determination of the total organic chlorine. The method is rapid, simple, and reasonably sensitive, but it is not specific for DDT. The sample is extracted with ethyl ether and Skellysolve B, the fat

in the extract then is saponified and removed from the mixture, and the aqueous filtrate extracted and subjected to a determination of chloride ion by any standard procedure. The amount of DDT is calculated by multiplying the amount of chlorine by 2. B.H.W.

99. **Recovery of Lactic Acid from Dilute Solutions.** A. A. Dietz with E. F. Degering, Purdue Univ., Lafayette, Ind., and H. H. Schopmeyer, Amer. Maize-Products Co., Roby, Ind. Indus. and Engin. Chem., Indus. Ed., *39*, 1: 82–85. Jan., 1947.

Lactic acid of varying degrees of purity may be obtained by solvent extraction, steam distillation and crystallization of its salts. The recovery of lactic acid by passing vapors of an alcohol through a partly concentrated lactic acid solution previously has been reported. A pure grade of acid may be prepared by the hydrolysis of an alkyl lactate. In the present investigation a study was made of the recovery of lactic acid as an ester directly from dilute solutions. The acid is converted to an ester and is extracted with a solvent in which it is preferentially soluble. Certain chlorinated hydrocarbons were found to be selective solvents. The preparation of ethyl and propyl lactates with 1,2-dichloroethane as the solvent is described. The esters can be purified to any desired degree by distillation. B.H.W.

100. **Applied Ultraviolet Spectrophotometry of Fats and Oils.** B. W. Beadle, Res. Lab., American Meat Institute, Univ. of Chicago, Chicago, Ill. Oil and Soap, *23*, 5: 140. May, 1946.

The application of spectrophotometry to the analysis of fats and oils is described. It is especially useful in studying the changes in the double bond systems of fatty acids. The method is applicable to routine analytical work in connection with the processing of oils as well as to academic studies on the composition of naturally occurring fats and oils. The determinations of small amounts of fatty acids with two or more double bonds or of small amounts of conjugated bonds are possible by this method because of its high sensitivity. J.L.H.

101. **The Oxidation of Methyl Oleate. I. The Preparation, Properties and Reactions of Methyl Hydroperoxido Oleate.** C. E. Swift, F. G. Dollear, and R. T. O'Connor. So. Regional Res. Lab., New Orleans, La. Oil and Soap, *23*, 11: 355. Nov., 1946.

The above work was designed to test reports in the literature that methyl hydroperoxido oleate is a product in the oxidation of methyl oleate. Methyl hydroperoxido oleate was separated by low temperature fractional crystallization from partially oxidized methyl oleate. Certain characteristics of the original hydroperoxido and its reaction products are described which "lend

definite support to the view that the first oxidation product of methyl oleate is a mixture of 8- and 11-hydroperoxido octadecenoic acid, at least under the conditions employed, *i.e.*, oxidation under the influence of ultraviolet light or reaction at temperatures up to 60° C."   J.L.H.

102. **Flavor Reversion in Soybean Oil. II. The Effect of Atmospheres of Different Oxygen Concentrations on the Flavor Reversion of Soybean Oil.** CALVIN GOLUMBIC, C. J. MARTIN, AND B. F. DOUBERT, Dept. of Chemistry, Univ. of Pittsburgh, Pittsburgh, Pa. Oil and Soap, *23*, 11: 360. Nov., 1946.

Samples of soybean oil were treated with slow-moving streams of oxygen, tank nitrogen containing 0.5% oxygen, or nitrogen purified by passage over heated copper turnings. The samples were maintained in a water bath at 45.5° C. in Petroff culture flasks. A 250-watt G.E. reflector-drying lamp was placed 3 in. directly over the flask and allowed to act during the flowing of the gas.

It was found that the oxidation rate of soybean oil could be varied over a considerable range without influencing the organoleptic evaluation of the degree of inversion. Even the low rate of oxidation attained by the use of purified nitrogen failed to influence the tendency to revert. When low oxygen concentrations were used, the typical grassy reversion flavor was accompanied by a disagreeable and persistent drying after-taste not readily detectable in the soybean oil reverted in air or oxygen. Reversion under nitrogen ocurred at very low peroxide values.   J.L.H.

103. **Flavor Reversion in Soybean Oil. III. The Preparation and Flavor Characteristics of a Simulated Soybean Oil.** CALVIN GOLUMBIC, A. I. SCHEPARTZ, AND B. F. DAUBERT, Univ. of Pittsburgh, Pittsburgh, Pa. Oil and Soap, *23*, 12: 380. Dec., 1946.

Fatty acids were prepared from Neofat, olive oil, cottonseed oil, and linseed oil. The fatty acids were mixed in the proportion in which they occur in soybean oil and were esterified with glycerol. The resulting simulated soybean oil was tested for flavor inversion, a defect common in soybean oil. The flavor produced in the simulated oil by heat and light treatment was distinctly different from the flavor appearing in soybean oil subjected to the same treatment. The results "tend to indicate that the ordinary fatty acid constituents of soybean oil are not entirely responsible for the flavor characteristics of reverted soybean oil. Likewise the hypothesis that linolenic acid is the sole causative agent does not appear likely although it is possible that this acid contributes to the flavor instability of soybean oil." J.L.H.

104. **Evaluation of Tests for Rancidity in Edible Packaged Oils.** JOHN E. W. McCONNELL AND W. B. ESSELEN, JR. Food Tech. Dept.,

Mass. State College, Amherst, Mass. Oil and Soap, *23*, 12: 369. Dec., 1946.

In this study oils were stored in sealed containers and later subjected to several tests in order to evaluate the reliability of these tests for quantitative measurements of the extent of rancidity (oxidative). The organoleptic test was found to be the most satisfactory method for determining the quality of corn and cottonseed oils which had been aged in sealed containers.

A high storage temperature or the presence of air influenced the fading time of methylene blue. In sealed containers the aldehyde formation in oils was very slow despite extensive development of rancidity. In oils exposed to air, the aldehydes increased. The results obtained on the changes in film pressure of monomolecular film of corn or cottonseed oil indicate that the change in pressure is not wholly dependent on the organoleptic quality of the oil but is influenced by the oxidation products formed in the presence of excess air.

The oils aged in the dark at 100° C. developed rancidity rapidly, whether stored in sealed containers or exposed to air. Little color change took place in the oils in sealed containers; those exposed to air darkened at first, after which bleaching occurred. Samples exposed to sunlamps at 38° C. deteriorated rapidly in organoleptic quality, whether exposed to air or not. Bleaching occurred in the sealed tubes whereas exposure to air slowed up this action appreciably. This indicates that light is the main factor in fading, while the darkening is caused by the excess oxygen and is accelerated by heat. The implications of these facts in the chain of reactions resulting in rancidity are discussed. The induction period of oils stored in sealed containers was found to be dependent upon the original peroxide value of the oils which, in turn, was influenced by exposure to light. Exposure to light resulted in destruction of peroxides. The chlorophyll value of an oil was found to be governed primarily by exposure to light rather than to its organoleptic state. Its general use is not regarded as a reliable test for rancidity. J.L.H.

105. Cleaning Procedure for Babcock Test Bottles. C. W. RINK. Canad. Dairy and Ice Cream Jour., *26*, 1: 30. Jan., 1947.

Calcium sulfate film in Babcock test bottles can be eliminated with a concentrated solution (30%) of caustic soda. The Babcock bottles are filled with the caustic soda and heated to boiling in a water bath for 30 min. The bottles are left in the bath for another 30 min. after the heat has been turned off. The caustic soda then is emptied and the bottles rinsed with water. If a haze remains, it can be removed with dilute hydrochloric acid or full strength vinegar. The 30% caustic soda can be reused for about three treatments. H.P.

CONCENTRATED AND DRY MILK; BY–PRODUCTS

106. Milk Powder Production Quality and Marketing. H. F. GEORGE. Canad. Dairy and Ice Cream Jour., *26*, 1: 68. Jan., 1947.

Good powdered milk has the qualities of any other form of milk. The ice cream and dairy industries will use considerably less powder in the near future than they recently were using. Powder used for standardizing will be replaced by fresh skim milk. Some extra solids for ice cream mix and for coffee cream will be needed, plus small amounts for standardizing purposes. The largest users of powdered milk will be the bakers. They will have to be educated to use more powdered milk. The solution to the marketing problem is to sell more powder to the bakers, both through personal contact and practical demonstrations, and to publicize the products for their high nutritional values.   H.P.

FEEDS AND FEEDING

107. Methods of Making Potato Silage and Tests of Its Feeding Value for Dairy Cows. J. B. SHEPHERD, T. E. WOODWARD, AND C. G. MELIN. U. S. Dept. Agr. Tech. Bul. 914. 14 pp. May, 1946.

Feeding trials with several lots of potato silage, ensiled without preservative, with salt, with ground corn, or with varying amounts of mixed orchard grass and clover hay are reported. Best results were obtained when potatoes were ensiled with hay; such silage was very palatable and cows made good gains on it and maintained milk production. Ensiling potatoes alone or with salt or corn meal did not prove practical. Raw chopped potatoes also were fed with satisfactory results when the allowance was not more than 4 lbs. daily per 100 lbs. liveweight. When the quantity of potatoes is small, they can be fed raw to best advantage. Potatoes can be preserved satisfactorily as silage along with 20 to 25% of good quality hay or may be put into the silo along with corn or other green crops. However, not more than 500 lbs. of potatoes should be used for each ton of green crop; a crop such as corn should be well matured; hay crops should be wilted to not more than 60% moisture. Tower silos should be well reinforced because of the heavy weight of potato silage. Such silage should be fed after milking and along with other roughage of good quality so that the ration will contain sufficient fiber, fat, minerals, and carotene.   J.G.A.

FOOD VALUE OF DAIRY PRODUCTS

108. The Digestibility of Fats—A Correlation of Experimental Data. KARL F. MATTIL, Res, Lab., Swift and Co., Chicago, Ill. Oil and Soap, *23*, 11: 344. Nov., 1946.

Reported data in the literature on the digestibility of fats fed to human adults, human infants, and white rats have been subjected to statistical

analysis and correlation coefficients calculated. A positive correlation (+ 0.77, calculated for 16 fats) exists between digestibilities found in human adults and those found for corresponding fats in albino rats.

The correlation coefficient for the relationship between digestibility coefficients and stearic acid content of fats was found to be negative (− 0.80 for human adults for 40 fats; − 0.77 for human infants for 16 fats; − 0.86 for albino rats for 26 fats). The amount of saturated acids of 18 carbon atoms or more that a fat contains is the chief limiting factor of its digestibility.

The coefficients of correlation for the relationship between digestibility and melting point are not as high as those for digestibility and stearic acid content, being − 0.66 for human adults for 37 fats and − 0.42 for albino rats for 24 fats. This lesser degree of correlation is due to the fact that the melting point is partially a function of the amount of long chain saturated acids.

<div align="right">J.L.H.</div>

109. **The Rôle of Various Substances in Stabilizing Animal Tissues.** G. O. BURR, W. O. LUNDBERG, AND J. R. CHIPAULT, Div. of Physiological Chemistry, Univ. of Minn., Minneapolis, Minn. Oil and Soap, *23*, 12 : 382. Dec., 1946.

The diet exerts an important influence on the oxygen uptake of body fat. The fats are influenced by their fatty acid composition and the presence of antioxidants or prooxidants. The most clear-cut demonstration is obtained by feeding or withholding tocopherol to rats. Tocopherols were found to differ among themselves in their effect on the keeping time of body fat. The alpha and beta form are twice as effective as the gamma form when fed to rats. The gamma form is several times as effective as an antioxidant as the alpha form when added directly to rendered body fat.

The type of fat in a purified diet was found to be very important. Butterfat was much more effective than lard in increasing the keeping quality of body fat of rats.

<div align="right">J.L.H.</div>

110. **The Rôle of Proteins in Animal Nutrition.** H. C. SCHAEFER, Ralston Purina Co., St. Louis, Mo. Oil and Soap, *23*, 12 : 375. Dec., 1946.

The rôle of proteins in animal nutrition is discussed. It is emphasized that adult ruminants are not as specific in their protein requirements as the single stomach or monogastric animals. The calf, in early life, is like the non-ruminants in that the rumen is undeveloped; hence it requires a better-quality protein in early life. The nutrition of small-stomached animals is concerned with amino acid nutrition or similar compounds, rather than with protein. More information is required on the nutrients or compounds furnished by proteins of high biological value and better methods are needed for the proper evaluation of proteins.

<div align="right">J.L.H.</div>

ICE CREAM

111. Rôle of Emulsifiers in Ice Cream Making. B. I. MASUROVSKY. Ice
Cream Trade Jour, *42*, 12: 72. Dec., 1946.

Egg yolk, monoglycerides, and diglycerides are being used as emulsifiers
for ice cream.   To evaluate a commercial preparation as an emulsifying
agent, the following test is suggested: Prepare a 15% sugar solution contain-
ing 10% butterfat. Introduce 0.25% of stabilizer and 0.25% of emulsifier
into the sweetened oil and water system.   Apply heat to insure the solubility
of the emulsifying agent. Pour the entire mixture into a graduated cylin-
der, agitate it for 2 min., and allow it to stand undisturbed for 10 min.
Examine the volume of free milk fat in the column and calculate the amount
of fat emulsified.   Compare it with a control mixture without an emulsi-
fying agent.

Ice cream stabilizers sometimes contain emulsifying material, and it is
advisable to follow directions given by the manufacturer of these products.
The trend seems to be toward the increased use of emulsifiers in ice cream
in an attempt to produce a higher quality product.   W.H.M.

112. Problems Arising from Increased Costs. RIDGWAY KENNEDY, JR.,
Abbotts Dairies, Philadelphia, Pa. Ice Cream Trade Jour., *42*, 10:
118. Oct., 1946.

During the war years the cost of ingredients used in the manufacture of
ice cream showed a marked increase. Costs such as delivery, sales, and
merchandising have been held in check because of increased volume of sales
and government restrictions. Production costs have risen because of in-
creased labor costs and the type of labor available. After price controls
were removed, the ice cream manufacturer could raise his price to offset in-
creased costs. During the past year manufacturing overhead costs have
been favorable, due to increased volume. However, they will go up if
volume begins to go down.

Should the ice cream manufacturer go back to daily delivery and increase
the number of flavors carried in stock, or spend excessive amounts of money
to get new business, costs are sure to rise. In order that sales volume may
be maintained, ice cream manufacturers might well consider the expenditure
of money on advertising campaigns designed to increase consumer acceptance
for ice cream. Money put into state and national nutrition programs will
tend to increase per capita consumption of ice cream and by so doing, oper-
ating costs may be reduced. New competition from within and without the
ice cream industry will make it necessary for ice cream manufacturers to
make decisions based on sound business judgment.   W.H.M.

113. Preserved Fruits—Preserve Flavor. F. I. Hutchins, Hutchins Advertising Co. Ice Cream Field, *48*, 6 : 30. Dec., 1946.

The manufacture of fruits, nuts and flavors represents a large industry which caters to the ice cream manufacturer. The trend is away from highly colored and extract-flavored products. Processed (*i.e.*, heated) flavors and fruits are considered more sanitary than fresh or cold packed fruits or "dry nuts". W.C.C.

114. Causes of Shrinkage in Ice Cream Making. B. I. Masurovsky. Ice Cream Trade Jour., *42*, 9 : 58. Sept., 1946.

Large air cells, heat shock due to sudden change in the temperature of ice cream cabinets, large ice crystals, utilization of certain types of milk solids such as those present in frozen cream, use of certain types of cocoa, improper blending of ingredients, and subjecting the ice cream to a wide range of temperature changes in storage are some of the causes of shrinkage. W.H.M.

115. Factors Affecting Shrinkage. R. J. Ramsey, Ramsey Laboratories, Cleveland, Ohio. Ice Cream Trade Jour., *42*, 12 : 46. Dec., 1946.

Shrinkage in ice cream may be affected by the following factors: (1) The destabilizing effect of freezing upon the colloidal suspension of proteins surrounding each air cell. (2) Low viscosity in the frozen ice cream, which may be influenced by ice crystal structure, sugar, type of freezer, and condition of the ice cream. (3) Air cell structure (fine air cells actually cause ice cream to shrink more readily than large air cells). (4) The use of dry ice. (5) The use of untreated paper in ice cream cans, boxes, or cartons. (6) Freezer operation. More shrinkage occurs in ice cream that is frozen too stiff and too dry at the freezers. (7) Air pockets due to poor filling practices. (8) High overruns. (9) Increased air circulation over cans. (10) Composition of ice cream. High butterfat, high sugar, excessive amounts of corn sirup, and the use of eggs seem to increase the tendency for ice cream to shrink. W.H.M.

116. Planning the Modern Ice Cream Plant. John W. Farley, Sales Engineering Dept., Cherry-Burrell Corp. Ice Cream Field, *48*, 6 : 18. Dec., 1946.

A consideration of small to medium combination milk and ice cream plants is presented. The general over-all purpose of planning such a plant is "To process the maximum quantity which can be sold of the highest quality products at the lowest possible cost".

The following factors are considered as important in the order listed: (1) amount of raw material to be handled, quantity of finished products to

be produced, as well as personnel and time available for required operations; (2) quantity of tools and equipment required, as well as expected methods of processing and handling; (3) location of building; (4) size and type of building to be constructed; (5) type of construction to be employed; (6) basis for deciding room arrangement in building; and (7) smallest number of changes which must be made in arrangement of equipment without disturbing the original plan. The necessity of surveys and reliable estimates of expected expansion are emphasized. By considering plant operation time schedules, equipment best suited for efficient operation can be selected. Flow plans are discussed from the point of view of efficiency in processing, packaging and storage. In plant layout it is highly desirable to have at least three sides of the building accessible. The use of a building accessible from only one side "almost invariably means a rather inefficient layout".

W.C.C.

117. **Bulk-Gallon Sales Spreading.** ANONYMOUS. Ice Cream Trade Jour., *42*, 10 : 96, 180. Oct., 1946.

The sale of bulk ice cream in single gallon containers for home use, which was started in March, 1946, by the Breyer Ice Cream Company in the Harrisburg, Pa., area, now has been expanded to other areas. Other ice cream companies now sell bulk ice cream by the gallon for home use. A label is placed on the container showing the retail price and how to store and dip the ice cream. Dealers are taking a 26% markup on this item.   W.H.M.

118. **Trends in Ice Cream Advertising and Sales.** E. L. WALKER, Arden Farms, Los Angeles, Calif. Ice Cream Trade Jour., *42*, 12 : 60. Dec., 1946.

Selling is an art, not a science. It is simply common sense and sound, fast thinking applied to business problems. The trend is away from teaching salesmen too much theory. Show them successful principles in action. Give them facts. Teach them to deal with specific situations. Train them in all phases of operation, including making mixes, freezing, novelties, and delivery. They should be familiar with restaurants, hotels, food markets, and drive-in markets. Ice cream manufacturers should see that their dealers play fair with the public and strive to give them good value for their money. Better package identification in cabinets also is helpful in getting better consumer acceptance. The trend is toward better balanced ice cream advertising campaigns, employing all media such as radio, newspapers, billboards, deluxe boards, point of purchase, car cards, and direct mail.

New possibilities for profitable ice cream merchandising are opening up every day. Some of them are: frozen food stores, vending machines, dairy departments, ice cream cabinets in apartment houses, complete frozen meals

with ice cream, complete cooked meals with ice cream, air lines, home and farm deep freeze unit sales, push carts, retail trucks, bicycles and boxes, modern power scooters, liquor stores for package sales, theater lobbies, and cabinets in drugless drug stores and in regular drug stores other than at fountains. Packaged sales, cups, and bars are becoming more popular; frosty malts also are on the upswing. W.H.M.

119. **Super-Market Merchandising.** VINCENT M. RABUFFO. Ice Cream Trade Jour., *42*, 12 : 34. Dec., 1946.

The anticipated entry of food chains and independent food stores into the ice cream marketing picture obviously is underway in all sections of the country. A striking example of how one well known wholesale ice cream manufacturer has reached out for important super-market ice cream volume is the case of the Bettar Ice Cream Co. of Baltimore, Md. The company supplies cabinets and the necessary promotional and advertising signs to 50 stores. The only item sold is the "Luxury Pint", which retails at 30 cents. The insulated bag for carry-home sales is an essential part of the program. A cardinal point in the sale of ice cream through food chains is that the ice cream be sold and merchandized through a department separate from that which sells frozen foods. W.H.M.

120. **Modern Ice Cream Store Planning.** DON MACK, Weber Showcase and Fixture Co., Los Angeles, Calif. Ice Cream Trade Jour., *42*, 12 : 39. Dec., 1946.

The six basic layouts for retail ice cream stores include the straight counter, the horse shoe counter, the island arrangement, the full service, the self-service, and the drive-in. Successful store operators follow these rules: (1) Select a store on the shady side of the street in the afternoon, and on the right side of the street on the way home from the heart of town. (2) Select a location where there are at least 300 families within a radius of 10 blocks. (3) See that the floor is attractive, of a usable surface, and that the ceilings and walls are of a light finish. (4) If you are in the same room with other merchants, insist upon the same side of the room that the staple items are merchandised from. (5) Select fixtures which will provide the greatest convenience to your customers, and provide the best working conditions for your employees both from the standpoint of speed and personal comfort. (6) Buy your supplies from wholesalers and jobbers who have a reputation for handling only the best. (7) Send announcements by mail to the 300 families in your neighborhood 3 or 4 days in advance of the opening of a new store, and at frequent intervals when you are going to offer particular flavors or special dishes with a varied appetite appeal. (8) Keep your store and equipment spotlessly clean and never under any circumstances allow a

customer to leave your store dissatisfied, even if you have to take a temporary loss.

These rules do not apply in country towns with regard to location.   In the country the best locations are near the busiest stores and on the same side of the street.   W.H.M.

121. The Merchandising Power of Sanitation. GEORGE HENNERICH. Ice Cream Trade Jour., *42*, 10: 112. Oct., 1946.

The ice cream manufacturer and his dealers must assume responsibility for the sanitary service of ice cream, as people are more conscious of cleanliness than ever before.   The first step should be the development of sanitary routines at the soda fountain and ice cream departments through which ice cream is sold to the public.   Sterilization of glassware between each use, clean dispensers, clean towels, use of dipper pads, and clean water in the dipper wells are routines which should be done correctly.   The motto of every retailer should be "Be Clean, Keep Clean, Serve Clean".   W.H.M.

122. Glorifying the Pint Package. VINCENT M. RABUFFO. Ice Cream Trade Jour., *42*, 10: 102. Oct., 1946.

The Riviera Ice Cream Co. of California operates more than 30 stores and also sells to dealers.   It produces only pint packages and controls the retail price by billing the ice cream to dealers at the full retail price less a percentage discount which represents the dealers' markup.   Stores operated in Los Angeles are distinctively designed, handle only ice cream, and are operated by one girl.   A high butterfat ice cream containing about 60% overrun is sold for 35 cents per pint.   Jiffy insulated bags with dry ice are furnished to customers for a 5 cent deposit, which is refunded if the bag is returned five times for refills.   Ten different flavors are sold.   W.H.M.

123. Ice Cream on Retail Milk Routes. ANONYMOUS. Milk Dealer, *36*, 4: 42, 66. Jan., 1947.

The Adohr Milk Farms, Los Angeles, is successfully distributing ice cream on its retail milk routes.   The ice cream is carried on the delivery routes in refrigerator boxes.   These boxes hold 24 pints of ice cream and are refrigerated with 2.5 lbs. of dry ice.   The ice cream is packaged only in pints.   Vanilla and chocolate are the only flavors regularly packaged.
                                                                      C.J.B.

124. The Ice Cream Industry and Frozen Foods. VINCENT M. RABUFFO. Ice Cream Trade Jour., *42*, 10: 100. Oct., 1946.

"The main ingredients of success in frozen food distribution are how well you are prepared to merchandise, sell, and service and remembering, all the

time, that frozen foods belong to the food business and yet are different than anything heretofore seen in the food industry." Actually, the frozen food business is six different businesses representing fruit, vegetables, fish, meat, poultry, and food specialties. It is a separate business from ice cream making and to be successful it must be operated as such. Any ice cream manufacturer going into the frozen food business should plan for all-year service and merchandising, but he also should have a separate organization for that purpose.   W.H.M.

MILK

125. **Some Angles on Leveling Milk Production.** C. W. PIERCE, Pittsburgh District Dairy Council, Pittsburgh, Pa. Milk Indus. Found. Assoc. Bul., *39*, 3: 56–68. Jan. 3, 1947.

Increased milk production during the fall months will be needed for several years due to population increases, prospective high demand, and larger business activity associated with good incomes. A large recurring fall shortage is likely to result in enlarging a city milk shed to the point where later conditions might unduly reduce the price of milk to the producer. It is to the interest of the producer to avoid severe shortages of milk. Better feeding and care will help, but the greatest leveling effect comes from a change in the breeding program. Spring freshening continues to predominate because farmers think such milk is produced more cheaply, even though cost records apparently show that fall freshening produces lower cost milk. A survey of several hundred farmers in Pennsylvania showed many believed fall and winter costs are higher than spring and summer costs by $1.09 per 100 lbs. It is concluded that an incentive fall price of less than $1.00 over the spring price would not greatly increase fall milk production. A higher class I seasonal price of the equivalent of 2 cents per quart initially, with perhaps 1 cent after enough production is obtained, is favored by the author over the "base rating" plan.   E.F.G.

126. **Off-Flavored Milk Due to Production Methods.** C. W. ENGLAND, Highland Dairy Farms, Washington, D. C., and Baltimore, Md. Milk Dealer, *36*, 4: 118–120. Jan., 1947.

The causes of off-flavor in milk are discussed. The discussion is summarized as follows: Off-flavored milk due to production methods can be avoided by eliminating the cause. Avoid feed flavors by bringing the cows off pasture-type feeds (or weeds) 3 to 7 hr. before milking. Strong flavored feeds, fed in the barn, should be fed after milking. Prevent flavors due to bacterial growth by practicing proper methods of sanitation and cooling. Eliminate off-flavors due to chemical composition changes by eliminating the guilty cows from the milking herd. Last, keep foreign materials out of milk.

Don't lose sight of the fact that people aren't going to drink milk unless they like it.   C.J.B.

127. **A Survey of Milk Bottle Costs, Disappearance and Trippage.** J. M. McAIRTY. Canad. Dairy and Ice Cream Jour., *26*, 1: 54. Jan., 1947.

The rate of new glass purchases varies roughly in proportion to population; it is quite probable that the length of delivery routes also is a factor. Other factors which may throw the average out of line are: (1) faulty equipment in one or more of the larger plants, (2) disregard for 5-cent deposit charge, (3) carelessness in handling bottles, and (4) costly system of exchanging bottles between dairies.

The 5-cent deposit decreased the rate of purchase of new bottles and led to the resurrection of used bottles from cellars and back yards. In 1945 in 13 plants in eight cities, the average number of trips per bottle was 5.8 and the average disappearance rate 1.72%. The universal bottle has reduced breakage and loss and has reduced expenses by elimination of sorting and exchanging bottles.   H.P.

128. **The Treatment of Cream.** TRYGVE LANGSLET, Malkeforsyningen, Oslo. Nordisk Mejeri-Tidsskrift, *12*, 7: 129–132. 1946.

I. The sanitary treatment of the cream.

The methylene blue test alone is insufficient for good control of the quality of milk and cream. Milk containing millions of bacteria of the type F3 (related to *Bacterium coli* and *Bacterium fluorescens liquefaciens*) has had a reduction time of more than 5.5 hr. by the methylene blue test. Lactic acid bacteria reduce methylene blue but do not grow on peptone agar; with the majority of the foreign bacteria the opposite is true. A combination of the methylene blue test and plate count on peptone agar gives an excellent evaluation of the bacteriological quality of the milk. A determination of the coli count in the milk received ought to be made at the same time.

Control when the milk is received is not enough. Samples for bacteriological control should be taken of the milk on its way through the whole pasteurization system, and from the moment the cream leaves the separator the control should be continued so the bacteriological quality of milk and cream always is known at any place. Small sampling valves can be placed in the system before and after possible sources of contamination.

A higher pasteurization temperature for the milk from which the cream is taken has been used with good results, as well as fast cooling and low storage temperature.

II. The treatment of the cream in order to get as high a viscosity as possible.

The treatment has been adapted from that of Henning and Dahlberg. The best results are obtained by cooling the cream slowly and to as low a temperature as possible after the pasteurization. The cream is heated to

between 75.2 and 91.4° F. Above or below these temperatures almost no effect in the viscosity is obtained. The best effect is obtained between 80.6 and 86° F.

A method similar to this one has been used in practice, a different one for cream with different fat content. After pasteurization the milk is cooled to 37.4° F. (not absolutely necessary but the best result is obtained by this) and is heated again to separating temperature. The cream is treated in special vats. Cream containing 35% butterfat or above is separated at 104–111.2° F., cooled to 37.4–39.2° F. in 1 hr., and remains at this temperature 1 hr. Next it is heated to 82.4° F. in 50–60 min. by warm water in the jacket under continuous stirring. Immediately after reaching this temperature the cream is cooled to 46.4° in ¾–1 hr. The cream is drawn in cans and placed in ice for further cooling.

Cream containing 30% butterfat is separated at 95–100.4° F., cooled to 35.6° F. in 1 hr., and kept at this temperature 0–1 hr. before heating to 82.4° F. in 90–110 min. (slow heating). The difference in the temperature between the warm water in the jacket and the cream in the vat must not be more than 18° F. Cooling to 46.4° F. in 1 hr. follows.

Cream containing 20% butterfat or less is separated at 78.8–86° F., cooled to 35.6° F. in 1 hr., and remains at this temperature for 0–1 hr. before heating to 82.4° F. in 110–130 min. (very slow heating). The difference in the temperature between the warm water and the cream must not be more than 9° F. Especially in the beginning the heating must be very cautious. Likewise the cooling from 82.4 to 35.6° F. must be done slowly in 90–110 min., for instance, first by water from 82.4–68° F. and then by brine. The cream is stored in the vat at 35.6° F. until the next day.

The treatment must be adapted for every plant. Bacteriological control of the cream has to made constantly or the cream can be subject to a reduction in quality.

III. The treatment of cream in regard to making it suitable for whipping.

The most important requirements for good whipping cream are:

a. Through the whipping the biggest possible increase in volume shall take place.

b. The cream shall be of a high stiffness.

c. It shall remain stiff for several hours without falling together.

d. The cream shall have a reasonable whipping time.

e. Little leaking of serum, and preferably none, at least not the first hour after whipping.

In order to get a stable product the separating temperature must not be too low. Pasteurized milk may be separated at 95° F. (depends on the season) and give a stable product, but not below 86° F.

Whipping cream ought not to be diluted with skim or whole milk, but

with thinner or fatter cream. The temperature of whipping ought to be 44.6–46.4° F.

IV. The packing of cream for sale.

The best thing to do is to sell the cream in bottles or cartons. This way gives the smallest loss and is the most sanitary. T.K.

129. **How to Sell Dairy Products Again.** GEORGE F. BARBER, Abbotts Dairies, Philadelphia, Pa. Milk Indus. Found. Assoc. Bul., *39*, 5: 105–119. Jan. 20, 1947.

A discussion of the selection of routemen is followed by a detailed procedure for conducting a 4-day training school for routemen, using the Milk Industry Foundation training manual, "The Balanced Job", as a guide. The time is divided into not over 15% in lecture, at least 40% in training the men, and 45% in exchanging experiences and opinions of specific problems and cases. The various duties of a routeman are analyzed and definite methods of instruction and procedure are specified. It is recommended that routemen be relieved of route duties for the period of the course, as it cannot be given effectively after a day of regular route service. E.F.G.

130. **What Kind of Plans and Materials are Put to Most Effective Use by Home Service Route Salesmen?** EDWIN FUNK, Sheffield Farms Co., Inc., New York, N. Y. Milk Indus. Found. Assoc. Bul., *39*, 5: 99–104. Jan. 20, 1947.

What helps are furnished to the routeman will depend upon whether a "moderate base, high commission" or "high base, moderate commission" payment system is in effect. A simple natural approach to the problem is advised to get him to use these helps most effectively. A check list of 23 details in producing routemen's sales tools is given under three headings, *viz.*, general, bottle collars and hangers, and folders and circulars. E.F.G.

PHYSIOLOGY

131. **Preparation and Chemistry of Anterior Pituitary Hormones.** ABRAHAM WHITE, Dept. of Physiol. Chem., Yale University, New Haven, Conn. Physiol. Rev., *26*: 574–608. 1946.

On the basis of physiological evidence, apparently at least six recognized individual hormones exist, although there is some biological overlapping among certain of the anterior pituitary secretions. The four most highly purified anterior pituitary proteins are the lactogenic, the adrenotrophic, the growth, and the luteinizing hormones. The thyrotrophic principle has been isolated in highly purified form but has not yet been examined by rigid criteria of protein purity. The follicle-stimulating hormone awaits further purification. D.E.

132. **Mechanism of the Development of Obesity in Animals With Hypothalamic Lesions.** JOHN R. BROBECK, Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn. Amer. Jour. Physiol., *26*, 4: 541–559. Oct., 1946.

Experimental study has shown that the obesity of animals with hypothalamic lesions arises primarily from a marked increase in food consumption, sometimes accompanied by a decrease in locomotor activity and by a transitory depression of basal heat production. The extra food eaten by the animal constitutes a relatively large energy surplus, which the tissues dispose of by storing some of it and by oxidizing the rest to carbon dioxide and water. Since lesions of the hypothalamus induce these highly typical deficits, the hypothalamus probably normally participates in the maintenance of the over-all energy equilibrium; the control of food intake, work output and body temperature may be correlated and integrated within this portion of the diencephalon. D.E.

MISCELLANEOUS

133. **Possible Trends of Dairy Research in Canada.** J. A. PEARCE. Canad. Dairy and Ice Cream Jour., *26*, 1: 64. Jan., 1947.

Possible trends in post-war dairy research in Canada are: (1) the utilization of waste dairy products or dairy by-products as specialty foods, (2) the possibility of using dehydrated whey in baking, and (3) the use of continuously operated dairy equipment, such as continuous butter-making machines and the adaptation of this churn to Canadian composition requirements for butter. Keeping quality studies will have to be made on this butter and results compared with butter made from the conventional methods of manufacture. H.P.

134. **Insect and Rodent Control in Dairy Plants.** GEORGE C. DECKER, Entomologist, Ill. Natural History Survey and Ill. Agr. Expt. Sta. Milk Dealer, *36*, 4: 124–128. Jan., 1947.

In the control of insects and rodents, both preventive and remedial measures should receive thorough consideration. Plant location and construction are emphasized among the preventive measures. The discussion of insect control is based mainly on the use of DDT. It is pointed out that, because DDT is used under greatly varying conditions to control many kinds of insects, it is marketed in several forms. Each has its distinct uses, advantages, and disadvantages. Following is a list of the forms now readily available:

(1) Prepared DDT dusts, ready for use, are available in concentrations of from less than 1% to 10 or 15%.

(2) DDT dust concentrates, containing 25 to 50% of DDT, to be used by jobbers or growers for preparing dilute dusts.

(3) Wettable powders, which are similar to dust concentrates but contain a wetting agent, are intended for use in the preparation of sprays.

(4) Oil solutions of several kinds are available. Some for use as household fly sprays contain as little as 0.2 to 1% of DDT in refined kerosene. Others intended for household use on bedbugs, flies, roaches, etc., contain 5% of DDT in refined kerosene or other suitable solvent.

(5) Emulsion concentrates are solutions of DDT, an emulsifying agent, and a solvent. They can be mixed with water to make sprays.

(6) DDT bombs, or "aerosol" bombs, contain DDT dissolved in liquefied gas. These bombs are for use in homes and other enclosed places.

DDT is used either as space sprays or as residual sprays. The space sprays also contain some quick-acting agent and are highly effective. They are used extensively in enclosed places. With the residual sprays, the residue left on surfaces sprayed or painted with DDT suspensions, emulsions, or oil solutions containing 1 to 5% of DDT will continue to kill flies, mosquitoes, roaches, etc., for from 1–2 weeks to several months after the spray is applied. The use of a 5% DDT solution or spray to be applied at the rate of 1 gallon per 1,000 sq. ft. of surface is generally recommended. DDT is effective in some paints but decomposes rapidly in whitewash.

Rodent control is discussed from the standpoint of prevention as well as poisoning. In addition to the poisons usually used, the uses of Antu (alphanaphthylthiourea) and 1080 (sodium fluoroacetate) are discussed.

C.J.B.

135. Berlin Diary (Dairy Edition). HENRY I. TRAGLE. Milk Dealer, *36*, 4: 47–48, 100–106. Jan., 1947.

A description is given of the C. A. Bolle plant in Berlin, which in normal times handled from 150,000 to 250,000 liters of milk per day. The plant had manufactured butter, margarine, ice cream, skim and whole milk cheeses, and a variety of fermented milk products. In addition they had operated a recovery plant where various chemical by-products of milk, such as casein and milk sugar, were extracted from the milk waste. High-temperature, short-time pasteurization was used. A description is given of the plant layout and equipment. C.J.B.

136. Future Price Supports for Dairy Products. DON S. ANDERSON, Dairy Branch, Production and Marketing Admin. Milk Indus. Found. Assoc. Bul., *39*, 3: 49–55. Jan. 3, 1947.

The Act of 1941 and the Steagall Amendment require the support of the prices of certain agricultural products at 90% of parity for 2 years after

Jan. 1 of the year in which the war is officially declared "over". (Abstractor's note—on Dec. 31, 1946, President Truman officially declared the war "over", so this act now supports prices through Dec. 31, 1948.) Little direction was given the Department of Agriculture with regard to how the support was to be effected.

Three possible methods of price support are suggested: (1) Do little about production but dispose of surpluses as can be done best. (2) Put into effect a strong production and marketing program, using supports to encourage shifts in production in predetermined directions. This probably would mean higher support prices for some products and quotas for others. (3) Employ a minimum of controls and some incentives to prevent unmangeable surpluses. The author suggests that any adjustment in the dairy industry probably will be toward more milk production rather than less. E.F.G.

137. **How Efficient Is Your Creamery?** L. C. THOMSEN, Univ. of Wis. Natl. Butter and Cheese Jour., *37*, 12: 38. Dec., 1946.

Changing conditions in the creamery industry require careful evaluation of plant efficiency. A chart is given which may be used for systematic study of factory operations. Factors included are quality, manufacturing methods, personnel, operating losses, accounting, sales, purchases of equipment and supplies, and public relations. Each factor is evaluated by the answers to specific questions which call attention to the important phases of the factor under consideration. W.V.P.

ABSTRACTS OF LITERATURE

BOOK REVIEWS

138. Ice Cream Industry. Second Edition. G. D. TURNBOW, P. H. TRACY, AND L. A. RAFFETO. 654 pages. $6.00. John Wiley & Sons, New York. 1947.

This textbook on the manufacture of ice cream is entirely new and replaces an earlier book by Turnbow and Raffeto. The text covers the ice cream industry from its early history up to and including the war years. The book is well illustrated, contains 25 chapters of worthwhile information, and is understandable to the plant man who has had no technical training.

There are chapters dealing with history, classification, and recipes. The recipes (15 pages) are largely for various flavors used and for ice cream of different flavors, sherbets, ices, and fancy ice creams. Adequate directions also are supplied. Other chapters cover composition of mix, selection of milk products, and sweetening agents with their relative sweetness values.

A chapter of considerable size deals with stabilizers in which gelatins, gums of the kinds used in ice cream, and emulsifying agents are discussed. A short chapter is devoted to eggs in ice cream, and another to standardization of the mix. "Mix Preparation" is the title of a long chapter covering equipment use, standardizing of acidity, pasteurization, homogenizing, cooling, restandardization of off batches, making mixes in the vacuum pan, and coloring. Two chapters are devoted to freezing the mix by batch and both kinds of continuous freezers, overrun control, and factors affecting overrun. Flavoring ice cream is covered in Chapter 12, while other chapters deal with hardening ice cream and with packaging and delivery. Ice cream novelties, sherbets and ices, specialties, and the manufacture of fancy ice cream forms are well handled. Several excellent illustrations show how fancy forms are made.

Sanitary control, bacterial content, washing and sterilizing, food value, defects, and physical and chemical properties are discussed in detail in chapters devoted to these subjects. The business phases of ice cream manufacturing are covered in chapters devoted to merchandizing, plant costs, and records. The final chapters deal with mechanic phases such as refrigeration, steam and equipment, the testing of dairy products used in ice cream, and the testing of ice cream for fat and total solids. C.D.D.

139. Prices of Dairy Products and Other Livestock Products. FRANK A. PEARSON AND EDMUND E. VIAL. 154 pages. $3.00. Cornell University Press, Ithaca, New York. 1946.

This book contains the results of a study to determine the effect of monthly production, stocks, supply, price level, demand, and competitive

products on the monthly prices from 1920 to 1941 for each of twelve live-stock products: butter, cheese, evaporated milk, nonfat dry milk solids, casein, eggs, poultry, lamb, veal, beef, pork, and lard. Oleomargarine and cottonsed oil also are discussed because of their close relationship to butter and lard, respectively. Most of the variations in prices of livestock prod-ucts are explained on the basis of the general price level and the production, stocks, or supply of the different products. These factors accounted for 45 to 94% of the variation in the monthly prices. The importance of price level varied from month to month. For some products large production and low prices were associated, while in others high prices and high pro-duction were related. Stocks generally were related inversely to prices, but the effects varied widely from season to season and from product to product. Butter prices, for example, were more closely related to supply than to pro-duction. The commonly used measures of demand—national income, earn-ings of factory workers, and business activity—did not explain much of the variation in the prices after the effects of the price level and production, stocks or supply had been eliminated.

The book is well written, with most of the data presented in simple tabu-lar and graphical form. A 14-page abstract and summary contains con-clusions based on the principal findings for each product and a statement of the economic principles involved. This book should be a welcome addi-tion to the library of any person interested in the factors affecting the prices of dairy and closely related farm products. W.L.S.

140. **Bacterial Chemistry and Physiology.** JOHN R. PORTER. pp. 1073 + x. $12.00. John Wiley & Sons, Inc., New York, N. Y. 1946.

This book is a needed addition to the literature of fundamental bacteri-ology, as it summarizes material from very diverse sources and provides an excellent bibliography for further study of special points. Much of the material is such as to have considerable technological application. Of the ten chapters, those on growth and death of bacteria, effects of physical agents on bacteria, effects of chemical agents on bacteria, bacterial enzymes and bac-terial respiration, bacterial nutrition, and microbial fermentations should be of special interest to people in the dairy industry. F.E.N.

141. **Concise Chemical and Technical Dictionary.** H. BENNETT, Editor. pp. 1055 + xxxix. $10.00. Chemical Publishing Co., Inc., Brook-lyn, N. Y. 1947.

More than 50,000 items, compounds, or names are described or defined. For many chemical compounds the chemical name, synonymous names, semi-structural formula, molecular weight, color, form, specific gravity, melting point, and solubilities are given. The biological terms in general are incom-

pletely listed, and many of those listed are not defined as well as one might wish. The coverage of trade names and common trade terms appears to be unusually good and is one of the most desirable features of the book. The rules of nomenclature of organic chemistry adopted by the Council of the International Union of Chemistry in 1930 are summarized in some detail, and an extensive list of names and formulae of radicals occurring in organic compounds is presented. The 1934 report of the Nomenclature, Spelling and Pronunciation Committee of the American Chemical Society is reproduced by permission. Tables of Greek, mathematical, apothecary and miscellaneous symbols are given, as are weights and measures and temperature conversion scales. The list of indicators unfortunately lists those for pH, oxidation-reduction potential, and specific compounds all in one group. The diagraming of many of the important organic ring systems provides a useful summary.

While this is not a handbook in the usual sense of the word, it fulfills many of the functions of a handbook and provides considerable information, especially in the fields of chemistry and related topics, which is not included in the usual handbook.   F.E.N.

BACTERIOLOGY

142. **A Review of Micrococcus Enterotoxin Food Poisoning.** W. C. HAYNES AND G. J. HUCKER, N. Y. State Agr. Expt. Sta., Geneva, N. Y. Food Res., *11*, 4: 281. July–Aug., 1946.

A rather complete summary of the available information relative to the rôle of certain varieties of micrococci in causing outbreaks of food poisoning or gastroenteritis in humans is presented. Milk and other dairy products frequently have been involved as sources of the organisms. Much of the presentation deals with the characteristics and properties of the bacterial enterotoxin, which is the real cause of the symptoms found in those suffering attacks. An extensive bibliography is presented.   F.J.D.

CHEESE

143. **Building of Store Rooms for Cheese.** FRANK LAMBERTSEN. Nordisk Mejeri-Tidsskrift, *12*, 11: 221–230. 1946.

The modern store room for cheese must have a capacity large enough that it can hold 3–4 months' production; thus the cheese does not need to be sold in a period of low prices. A one-story building is the ideal, because cheese can be trucked easily from room to room. A one-story store room may be built in the basement and extended out under the area around the factory. The racks should be low enough that a man easily can reach the cheese

placed on the upper shelf. A sufficient space between the shelves and between the shelves, ceiling, and floor is of great importance for good working conditions, as are wide passages between the racks. No rack should ever touch the wall. The walls, ceilings, and floors should be smooth and without irregularities. For sanitary reasons, the walls should be faced with brick.

If a one-story store room cannot be built, the staircases and elevators should be placed conveniently. Likewise, the different rooms—the salting room, curing room, the cold cooler, and the sales room—should be located conveniently.

Much is dependent on the temperature and the humidity in the store room. Good insulation and a good heating and cooling system are of great importance. Heating is best done by circulation of warm water. Radiators are best placed on the walls. The cooling system can work with an evaporation temperature of 32° F. or a little above, and the humidity easily can be held at 80–85° F. without artificial humidification. A cooling system in which the air is circulated by a fan is best. It prevents water condensate on walls and ceilings and renews the air.

In store rooms for Blue cheese and Emmenthaler cheese the fan system cannot be used because of the high humidity wanted. Pipes in which a cooling medium is circulated can be placed under the ceiling and cold air will circulate over the cheese and keep it cold.

Both building and equipment must be of good quality.   T.K.

144. Process for Making Material for Use in the Manufacture of Process Cheese. HIGBEE WAYNE BRYANT (to Kraft Cheese Co.). U.S. 2,392,362. Jan. 8, 1947. (5 claims).

In the manufacture of the class of cheese including Limburger, brick and Camembert varieties, after the curd is matted, it is subdivided into chunks not materially more than 1 inch in thickness and then washed promptly to prevent re-matting. The surfaces are subjected to action of appropriate aerobic ripening organisms in an atmosphere suitable for proper development and the ripened chunks then consolidated by heat to form the finished cheese. Increased rate of ripening under conditions which "substantially prevent" formation of undesirable rind is claimed.   F.E.N.

145. Process for Making Cheese. ALAN E. FLOWERS AND ANDREW E. MERGET (to The De Laval Separator Co.). U.S. 2,415,239, Feb. 4, 1947. (4 claims).

A process for removing gas from cheese curd dispersed in whey and centrifuging out the curd as the heavier component by a continuous process is described.   F.E.N.

CHEMISTRY

146. The Component Fatty Acids of Buffalo Colostrum Fat. C. P. ANANTAKRISHNAN, V. R. BHALE RAO, T. M. PAUL, AND M. C. RANGASWAMY, Imperial Dairy Research Institute, Bangalore, India. Jour. Biol. Chem., *166:* 31–33. 1946.

Composite mixtures of colostrum fat from four Murrah buffaloes taken the first five days, the tenth and fifteenth days of lactation were analyzed for the usual physical and chemical fat constants. The refractive index and the iodine value gradually decreased, with a corresponding increase in the Reichert and saponification values. Fat from the first, second, third, and tenth days of lactation was subjected to detailed chemical analysis by ester fractionation. "The chief changes to be found were the gradual increase in the amount of butyric, myristic, and palmitic acids and a decrease in the amount of stearic and oleic acids, the decrease in the latter being more pronounced." The analytical data are summarized in two tables. A.O.C.

147. The Immune Proteins of Bovine Colostrum and Plasma. EMIL L. SMITH, E. R. Squibb and Sons, New Brunswick, N. J. Jour. Biol. Chem., *164:* 345–358. 1946.

Colostrum and the protein fractions derived from it were studied electrophoretically. A lactoglobulin possessing all the immune properties of colostrum could be isolated, and data are given to show that this globulin easily is distinguished from β-lactoglobulin. In two different trials this immune globulin comprised 55% of the total protein of colostrum drawn 1 hour post partum. "Immune activity has not been found in fractions free from this protein, and conversely the isolated protein accounts completely for the immune properties of colostrum . . . by the second day the composition of the colostrum begins to approach that of milk and the immune lactoglobulin fraction can no longer be obtained free of other proteins by the simple method described."

Some discussion is devoted to the relationship of the various immune proteins. While the colostrum globulin is similar to the globulin found in bovine blood serum, the two are not identical. A.O.C.

148. Isolation and Properties of Immune Lactoglobulins from Bovine Whey. EMIL L. SMITH, E. R. Squibb and Sons, New Brunswick, N. J. Jour. Biol. Chem., *165:* 665–676. 1946.

"The high levels of immunity generally present in colostrum have served to obscure the fact that immunity is also present in the later milk. Though present in small amount, the immune proteins occur regularly in the whey of normal animals.

"Electrophoretic analysis has shown that the immune lactoglobulins constitute about 10 per cent of the protein in normal bovine whey. During hyperimmunization the immune components may increase considerably, although this does not occur regularly.

"A method has been described for the isolation from whey of the euglobulin and pseudoglobulin in electrophoretically homogeneous form. Immune activity is associated with both of these proteins.

"The isolated proteins have been studied in the Tiselius apparatus at different pH values, and the proteins have been characterized by their isoelectric points, diffusion constants, absorption spectra, and other properties.

"Studies in the ultracentrifuge reveal that all of the isolated bovine immune proteins contain more than one component. The principal component (76 to 92%) has a molecular weight of about 180,000." A.O.C.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

149. Plastic Cream, Its Production and Uses. R. J. SPIERS, Abbotts Dairies, Inc., Philadelphia, Pa. Dairy Ind. Found. Assoc. Bul., *39*, 7: 189–192. Feb. 14, 1947.

The present methods of producing plastic cream were perfected and patented by Abbotts Dairies, Inc., and a suitable bowl assembly designed by the De Laval Separator Co. A number of factors must be controlled carefully if the quality of the product is to be right. Included are: (1) good quality raw material, (2) freedom from copper or iron contamination, (3) temperature control (170° F. for 15 min. after the first separation and the second separation carried out at 145° F.), (4) product cooled enough to be like ice cream as it is packaged—never so much that paddles are necessary for handling, (5) use of a cylindrical cardboard container similar to 5-gallon ice cream can, (6) cooling as soon as packaging begins, using 10–20° F. air blast, (7) storing at 0 to –10° F., (8) shipping in brine-refrigerated cars, (9) keeping the fat content between 79.5–80.5% (all testing must be done at time of packing and not on frozen cream).

A thorough study of the psychrophylic type of bacteria in this product is needed. Plastic cream may be used for any purposes for which 50% cream is used and for some additional purposes. Largest use is in ice cream, with cream cheese a close second. If more attention is not paid to quality, plastic cream will lose much of the trade advantage it recently has earned.

E.F.G.

150. Manufacture of Casein by Means of Gyratory Motion Applied to an Inclined Screen. EDWARD J. WENDT (to Hercules Powder Co.). U.S. 2,415,268, Feb. 4, 1947. (4 claims).

This patent describes a device consisting of a means for causing gyratory

motion to which is attached a closed pan provided with an inlet, a fine-mesh screen on the bottom but tilted from the horizontal, and an outlet in the pan from the highest point on the screen. A catch basin is placed under the pan to collect the liquid after the very fine particles have been separated from it.   F.E.N.

151. **Dairy Process.** GERALD C. NORTH AND ALVIN J. ALTON (to Beatrice Creamery Co.). U.S. 2,392,401, Jan. 8, 1947. (6 claims).

Powdered whole milk is made by first separating the whole milk to fluid cream and skim milk. The resultant skim milk is condensed and heated to 170–180° F. for 15–30 min. The cream is heated separately to 170–180° F. for 15–30 min., then combined with the treated skim milk portion and the resultant mixture dried to a powder.   F.E.N.

FEEDS AND FEEDING

152. **Factors Affecting the Enzymic Destruction of Carotene in Alfalfa.** H. L. MITCHELL AND S. M. HAUGE, Purdue University Agr. Expt. Sta., Lafayette, Ind. Jour. Biol. Chem., *164:* 543–549. 1946.

"Enzymic destruction of carotene in alfalfa leaves was retarded as long as the tissues remained turgid, but increased rapidly with wilting. Since the loss of carotene was very rapid when the cells had been ruptured or otherwise modified by freezing, it appears that cell permeability limits carotene destruction. Under field conditions, little loss of carotene occurs until wilting takes place.

"Soil fertility had no significant effect on the carotene-destroying activity of alfalfa leaves.

"As the plants approached maturity, there was a slight decrease in carotene-destroying activity.

"There were no consistent differences in the carotene-destroying activity of the four varieties of alfalfa studied.

"Enzymic destruction of carotene during field curing appeared to be greater than destruction by light."   A.O.C.

ICE CREAM

153. **Retension of Ascorbic Acid in Strawberries during Processing, Frozen Storage and Manufacture of Velva Fruit.** H. J. LOEFFLER, Western Regional Research Laboratory, Albany, Calif. Food Res., *11,* 1: 69. Jan.–Feb., 1946.

Prime, mature strawberries averaged 66 mg. of ascorbic acid per 100 g. on a sirup-free basis 3 months after freezing. Immature fruit averaged 91 mg. and over-ripe fruit 55 mg. on the same basis. Whole or sliced berries

frozen without sugar lost no ascorbic acid during freezing. With sugar the losses amounted to about 10–15%. Whole berries packed in water lost about 20%, while pureeing or flash pasteurizing of the puree prior to freezing reduced the ascorbic acid not more than 5%.

On short storage (2–3 months), the berries frozen without sugar lost about 12–15% of their ascorbic acid; those with sugar, less than 5%; purees with sugar, 12%; and purees without sugar, about 16%. On extended storage up to 15 months, the sugared and unsugared berries lost only an additional 5% of ascorbic acid. The sugared and unsugared purees lost about 10 and 15%, respectively.

Losses during defrosting were found to be greater than in freezing and holding, particularly if the defrosting was slow or if the products were held some time after defrosting. When sugared puree was made into velva fruit, the loss of ascorbic acid was less than 5%. Unsugared purees, however, lost 12% during the mixing and refreezing.   F.J.D.

154. Retension of Ascorbic Acid in Raspberries during Freezing, Frozen Storage, Pureeing and Manufacture into Velva Fruit. II. J. Loeffler, Glacier Packing Co., Sanger, Calif. Food Res., *11*, 6: 509. Nov.–Dec., 1946.

Essentially all the ascorbic acid was retained in raspberries during freezing and storage up to 4 months, and not over 25% was lost up to 28 months. Sugar was found of definite value in reducing even this small loss. In frozen raspberry purees, lack of sugar more than doubled the loss of ascorbic acid during 16 months of storage.   F.J.D.

155. Ice-Cream Freezer. Leroy H. Knibb. U.S. 2,416,326, Feb. 25, 1947. (15 claims).

The motor-driven freezer is intended for insertion into the ice compartment of a refrigerator. Provisions are made for discharge to the atmosphere of the air heated by the driving motor.   F.E.N.

MILK

156. When Electricity Is Used for Pasteurization Does It Fit in with Other Plant Operations. Israel Adams, St. Lawrence Dairy, Reading, Pa. Dairy Ind. Found. Assoc. Bul., *39*, 7: 177–179. Feb. 14, 1947.

The installation of an electrical pasteurizer presents no problems that cannot be corrected as readily as when installing any other system of pasteurization. The possibility of two or three small units to get the desired total capacity is suggested.   E.F.G.

157. Pros and Cons of Short-Time High-Temperature Pasteurization.
R. J. WINNING, Sheffield Farms Co., Inc., New York, N. Y. Dairy Ind. Found. Assoc. Bul., *39*, 7 : 180–188. Feb. 14, 1947.

The advantages include: (1) a double safety factor from automatic control and the flow diversion valves, (2) lower equipment cost for large plants, (3) less floor space, (4) regeneration savings, (5) less labor in cleaning, (6) easier to operate, (7) more uniform product, (8) elimination of trouble from thermophilic bacteria, (9) expansion at small cost, (10) less milk held too long in case of shut down, (11) lower milk losses, (12) fact that thermoduric bacteria may survive encourages cleaning up milk supply, and (13) equipment more easily sterilized.

Disadvantages are: (1) gasket trouble, (2) some products as buttermilk, sour cream, etc., do not process well, (3) possibility of freezing when a low temperature cooling medium is used, (4) flow diversion valve should be improved and body made of stainless steel.

Some suggestions for use include checking flow in both forward and diverted position, keeping the unit completely airtight for ease of cleaning, and checking the flow with both homogenized and regular milk when putting out homogenized milk.   E.F.G.

158. Experiences Using the S.T.H.T. on Milk, Cream, Buttermilk, Cocoa and Cottage Cheese.
MARTIN KLOSSER, Bowman Dairy Co., Chicago, Ill. Milk Ind. Found. Assoc. Bul., *39*, 7 : 167–179. Feb. 14, 1947.

Results from STHT as compared with a long flow method are as good from the standpoint of thermodurics, thermophilics, phosphatase test and cream volume, and better for the flavor of the milk. Seven units are operated in four plants of the writer's organization, with a total capacity of 105,000 lbs. of milk per hour. Both the Chicago Board of Health and the plant engineer frequently check the operation of the units in various ways. Sixteen hours with 75% regeneration seems to be the maximum length of run before loss in heat transfer rate requires disassembling and cleaning up the unit. Milk is heated to 161.5° F. for 16 sec. with clarification between regenerator and heater. Homogenized milk is heated to 170° F. for 16–18 sec., going from regenerator to homogenizer to clarifier to heater at 70° F. Cream is cooled in the cooling section. Thirty-six per cent cream takes about 20 sec. rather than 16 to pass through the holder. Skim milk for culture is heated with a second regenerator and heater to 185–190° F. When processing chocolate drink made from cocoa, a meandering retarder gives a 10 min. holding period at 180° F. Cocoa has a grinding effect on pumps, which need to be checked frequently. For cottage cheese 161.5° F. for 16 sec. is high enough; higher temperatures give curds which are too

fine. For cleaning, the alternate acid and alkali methods are used, followed by assembly and a sterilizing water rinse. STHT is used for all fluid milk, specialty products, and by-products, except sour cream and ice cream mix.

E.F.G.

159. **Some Cooking Qualities of Homogenized Milk. II. White Sauces.** ALICE M. TOWSON AND G. M. TROUT, Michigan State College, East Lansing, Mich. Food Res., *11*, 3: 261. May–June, 1946.

White sauces made with homogenized milk failed to incorporate added fats as well as when made with unhomogenized milk. As the amount of fat was increased, the difference became more pronounced. The viscosity of the sauces increased as the pressure used in homogenizing the milk was raised. Beaten sauces made with a rotary beater were smoother in texture and superior in flavor when homogenized milk rather than unhomogenized milk was incorporated into them.

F.J.D.

160. **Improving Milk Quality from Cow to Plant.** C. B. A. BRYANT, Johnson and Johnson, Chicago, Ill. Milk Dealer, *36*, 4: 49–54. Jan., 1947.

See Abs. 72, Jour. Dairy Sci., *30*, 3: A33. Mar., 1947.

161. **Bottle Capping Head.** CARL W. GOODWIN (to American Seal-Kap Corp.). U.S. 2,416,001, Feb. 18, 1947. (4 claims).

A capping head for use with a hood cap having a central diaphragm, a top wall, and a fluid marginal skirt to be folded around the outer surface of the bottle neck is described.

F.E.N.

162. **Method and Apparatus for Pasteurizing Liquids.** RAYMOND E. OLSON (to Taylor Instrument Co.). U.S. 2,415,304, Feb. 4, 1947. (10 claims).

The basic change involved in the usual high temperature–short time pasteurization process is the provision for raising the temperature of the heating fluid to a value above the predetermined level in response to diversion of the product when it fails to maintain the prescribed temperature.

F.E.N.

MISCELLANEOUS

163. **Federal and State Standards for the Composition of Milk Products.** ANONYMOUS. U. S. Dept. Agr., Bureau of Dairy Industry, Leaflet BDIM—Inf—45. 4 pp. Feb., 1947.

Federal, State and Territorial standards in force Jan. 1, 1947, are pre-

sented in tabular form, with explanatory footnotes in many instances. Data on minor products are not included.   F.E.N.

164. **Manufacture of Cream Products.** LLOYD K. RIGGS (to Kraft Foods Co.). U.S. 2,414,837, Jan. 28, 1947. (6 claims).

A material containing 80 to 95% milk fat is produced by adjusting cream to pH 3.8 to 4.8, heating to at least about 180° F., and then centrifuging the heated acid cream to break the original emulsion.   F.E.N.

165. **Sediment Testing Device.** BERNARD L. KINYON. U.S. 2,414,044, Jan. 7, 1947. (11 claims).

A portable sendiment tester using vacuum and compressed air for actuating the reciprocal plunger within the barrel is described. A measured quantity of milk is drawn into the barrel on the suction stroke and discharged through the filter on the pressure stroke.   F.E.N.

166. **Method of Removing and Concentrating Residue from Containers.** E. ROY ALLING AND HENNING A. TREBLER (to Rice & Adams Corp.). U.S. 2,418,063, Mar. 25, 1947. (11 claims).

A pre-rinse for a continuous can washer, in which two lots of detergent-free water are used consecutively and repeatedly until a "marketable concentration" of material such as milk is built up in the first of the two rinses, is described. The rinsed cans then are washed with water containing a detergent.   F.E.N.

167. **Can Dumping Mechanism.** CLAUDE H. ABBOTT. U.S. 2,413,900, Jan. 7, 1947. (5 claims).

A power-driven can dumping device for use with milk cans is described.
                                                        F.E.N.

168. **Defrosting Frozen Foods by High Frequency Heat.** W. H. CATHCART AND J. J. PARKER, National Bakery Division, The A & P Tea Co., New York, N. Y. Food Res., *11*, 4: 341. July–Aug., 1946.

Utilizing a 3-kilowatt high frequency unit made by the Federal Telephone and Radio Corporation, the authors were able to defrost frozen eggs, fruit, vegetables, and fish in cardboard containers in from 2 to 15 min., depending on the size of the package. This was accomplished without loss of quality, such as discoloration and flavor deterioration.   F.J.D.

176. Application of Sendroy's Iodometric Chloride Titration to Protein-Containing Fluids. D. D. VAN SLYKE AND ALMA HILLER, Hosp. Rockefeller Inst. for Med. Res., New York. Jour. Biol. Chem., *167*: 107–124. 1947.

The Sendroy iodometric method for chlorides is based upon the liberation and measurement of the IO_3 radical according to the following equation: $AgIO_3 + Cl^- = AgCl + IO_3^-$. The IO_3^- is measured by titrating against a standard thiosulfate solution.

"In the present application, the titrimetric procedure for protein-containing fluids is simplified by carrying out the reaction with $AgIO_3$ and precipitation of proteins simultaneously in a single operation, so that an entire analysis, including removal of the mixed precipitate and titration of the filtrate, can be carried through in about 6 minutes."

A table comparing this method and the nitric acid digestion method in the analysis of cows' milk is given. A.O.C.

177. The Determination of Iron in Biological Material. A. J. WOIWOD, Wellcome Physiological Research Laboratories, Beckenham, Kent (England). Biochem. Jour., *41*, 1: 39–41. 1947.

"A method for determining iron in biological materials with a high P : Fe ration is described. It is applicable over the range 0.5–10 μg. Fe/ml. All analytical manipulations, except the final centrifuging before colour reading, are performed in the crucible in which the sample has been ashed. Blanks are therefore kept at a minimum.

"The method has proved satisfactory with protein hydrolysates and cows' milk and may be suitable for other materials where phosphate interference is met."

A value of 4.7 μg. Fe/10 ml. is reported for raw milk. Twelve references are given. A.O.C.

178. Constant Pressure Oxygen Absorption Fat Stability Test. G. GILMONT, H. S. LEVENSON, AND L. W. ELDER. General Foods Corp., Hoboken, N. J. Oil and Soap, *23*, 8 : 248. Aug., 1946.

Details are given for the equipment and operation of the General Foods Method of determining fat stability. The method, essentially a modification of the Barcroft-Warburg procedure of Johnson and Frey, differs from the latter method in two respects: (a) The oxygen is absorbed under constant pressure and can be recorded volumetrically on a macro scale. (b) The induction periods can be evaluated graphically from the direct plot of the experimental data without further calculation. With a 2-ml. sample the induction periods were reproducible with a precision of 1 to 2% in most cases or a maximum variation of 5% in the most unfavorable cases.

J.L.H.

179. Interfacial Tension of Oil-Water Systems Containing Technical Mono- and Diglycerides. R. O. FENGE, So. Regional Res. Lab., New Orleans, La. Jour. Amer. Oil Chemists Soc., *24*, 2 : 49. 1947.

Technical mono- and diglycerides have wide industrial use as oil-soluble emulsifying agents in the manufacture of superglycerinated shortenings and margarine. Commercially available products are composed of mixtures of mono-, di-, and triglycerides. Known mixtures of mono-, di-, and triglycerides have been studied but little with respect to their power of lowering the interfacial tension at vegetable oil-water interfaces. Technical mono- and diglycerides were prepared for study. When both mono- and diglycerides are present in the oil phase, the interfacial tension is substantially a function of the monoglyceride content. A constant weight of a given monoglyceride preparation had practically an equal effect in lowering the interfacial tensions against water for peanut, cottonseed, and soybean oils. A concentration of 1% of monoglyceride in the oil phase was found to lower the interfacial tension at the oil-water interface by approximately one-half, and 6% lowered the interfacial tension to practically zero.

J.L.H.

FEEDS AND FEEDING

180. Effect of Dehydration on Enzymic Destruction of Carotene in Alfalfa. H. L. MITCHELL AND H. H. KING, Dept. of Chem., Kansas State College, Manhattan, Kans. Jour. Biol. Chem., *166*: 477–480. 1946.

As alfalfa cures in the field the enzyme lipoxidase is responsible for the destruction of a considerable part of the carotene. Peroxidase, another oxidative enzyme present in alfalfa, is inactivated by blanching prior to dehydration (mechanical drying). By blanching and dehydration both enzymes are inactivated and neither is regenerated during storage of alfalfa meal for 2 months.

"Blanching of alfalfa prior to dehydration did not increase the retention of carotene during storage. Carotene destruction during storage does not appear to be enzymic in nature." A.O.C.

FOOD VALUE OF DAIRY PRODUCTS

181. The Effect of Fat on the Absorption and Utilization of Galactose by the Rat. MARIE L. NIEFT AND H. J. DEUEL, JR., Dept. of Biochem. and Nutr., Univ. of Southern California, School of Medicine, Los Angeles, Calif. Jour. Biol. Chem., *167*: 521–525. 1947.

The present work confirms, to a large degree, that reported by Geyer *et al.* (See Abs. 358, Jour. Dairy Sci., *29*, 10: A164. 1946.) The investi-

gators conclude: "The percentage of ingested galactose which is excreted in the urine varies inversely with the percentage of fat in the diet."

"The fat effect appears to be independent of the type of fat at a 20 per cent level, since butter fat, corn oil, and cottonseed oil give essentially the same results. However, if the fat level is cut to 10 per cent, cotton-seed oil gives significantly lower urinary excretion values than either butter fat or corn oil."   A.O.C.

ICE CREAM

182. Shrinkage in Ice Cream. C. D. DAHLE, D. J. HANKINSON, AND J. A. MEISER, JR. Ice Cream Rev., *30*, 6 : 41. Jan., 1947.

The least amount of shrinkage was observed with ice cream stored in glass containers, followed in order by metal, paper paraffined inside and outside, paper paraffined on the inside only, paper paraffined on the outside only, and untreated paper. Shrinkage was greatly reduced when a cabinet temperature of $+5°$ F. was used as compared with $+10°$ F., irrespective of the type of container used. No shrinkage was observed in ice cream samples stored at -15 to $-20°$ F. Substitution of 30% of the cane sugar on a dry weight basis with sweetose, cerelose, Frodex, or honey increased the amount of shrinkage in the order named. When corn sirup was substituted for 30% of the cane sugar, less shrinkage was observed than when cane sugar alone was used. Pasteurization of the mix at 160° F. for 20 min. or 160° F. for 45 min. resulted in slightly less shrinkage than when pasteurization temperatures of 145° or 175° F. were used. Wet (incompletely frozen) ice cream was found to shrink more than that frozen enough to yield a dry appearing ice cream when drawn from the freezer. High homogenization pressures were found to increase shrinkage, with little or no relationship being observed between the homogenization pressures used and the dryness of the ice cream at the freezer.

Four different stabilizers showed little difference in the amount of shrinkage. Certain emulsifiers and egg yolk increased the dryness of the ice cream and increased shrinkage somewhat. No relationship was observed between the source of gelatin nor its Bloom strength and shrinkage. The use of superheated condensed milk resulted in less shrinkage in ice cream than when plain condensed milk was used. Butterfat from different sources gave variable and inconsistent results as related to shrinkage. The mix acidity and protein stability could not be correlated definitely with shrinkage. The addition of sodium salts to ice cream mix had little or no effect on the amount of shrinkage observed. The addition of calcium salts did accentuate shrinkage slightly. Presence of 0.4% free fatty acids in the mix increased markedly the amount of shrinkage observed in the ice cream. The use of dry ice was found to accentuate shrinkage, particularly with ice cream frozen in a continuous freezer.   W.J.C.

183. Manufacture of Powdered Ice Cream Mix. HARRY PYENSON. Ice Cream Rev., *30*, 8 : 54. 1947.

The composition, advantages, markets, and results of experimental studies dealing with the manufacture, storage, and use of powdered ice cream mix are discussed. Stabilizers of vegetable origin gave more satisfactory results than those of animal origin and improved the whipping properties of the mix when used in conjunction with an emulsifying agent, such as glycerol monostearate S. Addition of all of the sugar to the mix prior to condensing resulted.in a caramel flavor, reduced the capacity of the drying equipment, and interfered with the drying process. The addition of only 25% of the sugar prior to condensing is recommended. High quality dairy products processed in stainless steel equipment, high pre-heating temperatures, presence of antioxidants and low moisture content (below 1.5%) were essential to the production of powdered ice cream mix of good keeping quality. Such a product showed very little change in flavor for 8 to 12 months or longer.

A suggested procedure for the production of powdered ice cream mix is as follows: (1) Combine fluid milk and cream to give the desired ratio of fat to solids-not-fat, condense, and add 25% of the sugar and stabilizer, or add the sugar and stabilizer to the milk and cream prior to condensing. (2) Preheat to not less than 170° F. for 20 min., 180° F. for 10 min., or 190° F. for 5 min. (3) Condense to 30–36% solids, depending on whether stabilizer is added before or after condensing. (4) Homogenize the condensed mix at 150° F., using a pressure of 2,500 lbs. for the first stage and 500 lbs. for the second stage. (5) Preheat the homogenized mix to 150° F. before spray drying. (6) Spray-dry the mix to yield a product to contain not over 1.5% moisture. (7) Cool the powdered mix immediately. This will minimize cooked flavor and retard the development of stale flavor. (8) Add the remainder of the sugar. (9) Gas pack the powder in tins.

Powdered mix usually analyzes: Butterfat, 27–30%; M.S.N.F., 27–28%; sugar, 39.5–44%; stabilizer, 0.6–1.0%; and unspecified material, 2.75%. The powder may be reconstituted by adding it to cold water in the freezer and freezing immediately, but better quality ice cream resulted when the reconstituted mix was allowed to age for 24 hr. prior to freezing. W.J.C.

184. Improving Package Ice Cream. J. H. ERB. Ice Cream Rev., *30*, 8 : 44. March, 1947.

The challenge confronting the ice cream industry is to produce in factory-filled packages the same desirable characteristics the consumer associates with hand-dipped ice cream. Key factors in attaining this objective are: (1) Total solids content of the mix should be between 38.5–40%. (2) Use of high quality raw ingredients. (3) Thorough homogenization. (4)

Aging of mix for not less than 5 hr., irrespective of the stabilizer used. (5) Maintaining proper weight in all packaged ice cream. (6) Adoption of overrun standards well below those used for bulk ice cream. Freezers in battery operation should be checked to see that ice cream of the proper overrun is being delivered by each freezer. (7) Freezing the ice cream to a stiff dry consistency at the freezer and avoiding the use of long, small diameter lines in conducting the ice cream from the freezer to the filler head. (8) Hardening time not to exceed 6 to 8 hr.   W.J.C.

185. **"Sanitary Spoon Rest."** ANONYMOUS. Ice Cream Rev., *30*, 8: 43. March, 1947.

Sales resistance to tall fountain drinks on the part of the customers who do not like to put wet spoons on the counter when not in use may be overcome through the use of an ingenious paper spoon holder known as "Sanitary Spoon Rest". This device, manufactured and introduced in the Los Angeles area by Coast Curries Ice Cream Co., helps keep the counters clean, prevents sloppy serving of the spoon, and also is an effective medium for advertising.   W.J.C.

186. **Lower Costs in the Ice Cream Industry.** CARL H. ZANZOW, JR. Ice Cream Rev., *30*, 8: 48. March, 1947.

The objective of every ice cream plant to increase productivity and, simultaneously, reduce costs can best be achieved by the establishment of a complete production and engineering department, responsible only to top management. Functions of such a department are: supervisory training, plant layout and process improvement, work simplification and methods improvement, work measurement, employee training, planning and scheduling of operations, job evaluation, establishment of sound wage incentives, and cost control. A common understanding of the entire program is essential by all levels of management, the employees and their union, and can be achieved best by a series of group-participating conferences.

Results to be expected from a properly functioning production engineering program are: (1) proper plant layout and process flow, (2) simplified and standardized working methods, (3) establishment of fair and equitable work standards, (4) establishment of a fair and equitable base wage rate structure built upon a sound job evaluation procedure, (5) improved production scheduling throughout the plant, (6) increased employee earnings through the use of wage incentives, (7) reduction in unit costs by a greater output per man hour, (8) the control and elimination of material waste, (9) decreased labor turnover, (10) the establishment of standard costs, and (11) effective cost control reports to measure progress and point out weaknesses that can be remedied.   W.J.C.

187. Trends in Ice Cream Merchandising. R. A. PERRY. Ice Cream Rev., *30*, 8 : 45. March, 1947.

A program designed to surround ice cream with an atmosphere which will intensify its appeal and remove any barriers which might serve as obstacles to consumer acceptance must start with the manufacturer and carry through to the ultimate consumer.

The manufacturer has the responsibility of seeing that a uniformly high quality product, attractively packaged, is supplied at all times. The plant and delivery equipment must meet the most exacting demands of sanitation, orderliness, and attractiveness. The ice cream manufacturer must realize that he must follow his product through to the ultimate consumer. This involves closer cooperation between supplier and dealer. Sanitation at the point of sale is stressed as the phase of activity most deserving of immediate attention. The appearance of the store, its personnel, odors, cleanliness of the counter, seats, glasses, silverware, etc., all are factors which influence customer satisfaction.

Education of the dealer through actual visual demonstrations is presented as the most effective means of dealer education on the part of the ice cream manufacturer. Eleven concrete suggestions for making the dealer into an effective merchandiser for ice cream are presented. W.J.C.

188. Selling Ice Cream through Vending Machines. E. THOM. Ice Cream Rev., *30*, 7 : 41. Feb., 1947.

The use of vending machines for the distribution of ice cream opens up a new and virtually untouched market. Experience gained since 1940 by Miller Bros. in New York City should prove extremely helpful to any ice cream company contemplating entering this field.

The operation of vending machines demands the full-time attention of a separate department or a separate company. It cannot be handled successfully as a part-time job on the part of some individual within the organization. The ice cream served must be of unvarying high quality which will merit repeat sales, and flavors should be confined primarily to vanilla and chocolate. The nickel ice cream cup is the item which probably can be sold most successfully through vending machines, although the possible vending of ten-cent cups and other novelty items is being tried out in a limited way. Vending machines with a capacity of not less than 200 cups should be installed and these must be kept supplied with ice cream at all times. Service men should be paid in part on a commission basis to increase their interest in keeping the machines filled and in operating order. They should not be expected to make repairs on machines except for minor adjustments requiring 10 min. or less. Vending machines should be located with firms primarily interested in providing a service for their employees rather than with

firms interested in the commission which may be received as rental for the space and electricity provided. This method of distributing ice cream has the advantage of being a strictly cash business and serves to bolster sales during the winter months.   W.J.C.

189. The Need for Cooperation. C. J. PALMER. Ice Cream Rev., *30*, 7: 48. Feb., 1947.

Cooperation between manufacturers of ice cream and of soda fountain equipment can do much to make soda fountain operators appreciate more fully the importance of good equipment and the importance of providing outstanding service to their customers. Such cooperation will result in increased sales of both ice cream and soda fountain equipment, thereby working to the mutual advantage of both groups. The two groups should cooperate to the end that: (1) Soda fountain operation will be above any possible criticism from a sanitary standpoint. (2) Proper facilities will be provided for the cleaning and sterilization of all multi-use utensils. (3) The possibilities of expanded food service at the fountain will be brought to the attention of the operators. (Surveys have shown that offering both food and fountain service will result in greatly increased ice cream and fountain sales as well as a more profitable over-all operation.) (4) Fountain drinks of superior quality will be served as a result of use of proper equipment and education of the operator in the essential steps of correct carbonation.

W.J.C.

190. The Importance of Ice Cream in the Dairy Industry. R. C. SMELLIE. Canad. Dairy and Ice Cream Jour., *26*, 3: 37. March, 1947.

The value of ice cream can be illustrated best by its continued manufacture during the war and at a volume in excess of the 1939 production. An increase in population and a decline in cow and heifer population are making less milk available for ice cream in Canada today. The dairy producers have to be convinced that all the milk they produce will have a profitable outlet. Ice cream is officially recognized as a food in Canada and more publicity will be given to it when more ice cream is available.   H.P.

MILK

191. Milk Production Trends in the U.S.A. and Possible Competition from Canada. T. M. ADAMS. Canad. Dairy and Ice Cream Jour., *26*, 3: 59. March, 1947.

The sound position of dairying in the northeastern region of U.S.A. lies in the adaptability of the area to the economical production of an abundant supply of high quality roughage and pasture and its nearness to large populations of consumers of fluid milk. Cost of transportation and the quality

factors associated with the movement of milk over long distances have made for an economic barrier excluding midwestern fluid milk from New England markets. Competition from Canada may increase if the barriers which limit milk imports are removed or lowered. H.P.

192. Operation of Six-Day-Week Milk Delivery. A. GIGANAC. Canad. Dairy and Ice Cream Jour., *26*, 3: 66. March, 1947.

Windsor, Ontario, has successfully operated on a 6-day-week milk delivery for the past 4 years. Milk is not delivered on Sunday or on a holiday that occurs on Wednesday or Thursday. The management, employees, and the public find 6-day delivery more economical and more pleasant, and it simplifies delivery operations. Six-day delivery and plant work are not difficult providing good storage facilities and excellent facilities for raw milk are available. H.P.

MISCELLANEOUS

193. Pasteurization by Ultra-Violet Rays. E. CAPSTICK, H. HALL, AND F. K. NEAVE. Milk Indus., *27*: 8. Feb., 1947.

The German Prof. Lembke and Dr. Bayha have developed a process for pasteurization by ultra-violet rays. The optimum wave length for a bactericidal effect is 2537 Å. The effective penetration is restricted to approximately 1 mm. A sheet metal cabinet houses 28 quartz-tube mercury vapor lamps. Milk flows through quartz tubes on a three-pass system to form a continuous path 328 feet long. The milk in each bank of 50 tubes is irradiated by 12 mercury vapor lamps. Air temperature in the cabinet is maintained at 20–30° C. The unit was designed to operate at 1,200 l. per hour (260 gallons) but operates at the present time at about 140 gallons per hour. The equipment is easily cleaned by circulating cold water, followed by a warm alkaline detergent solution, then water acidified slightly with HCl, and finally clear cold water. The bacteriological tests made show that an exposure for 1.75 min. with the milk at 30° C. gave better results than milk held for 30 min. at 63° C. With poor quality milk coliform organisms survived in the irradiated milk. Many more investigations have to be made to determine the proper amount of radiation, temperature of milk and lamps, turbulence, the importance of such factors as aeration and deaeration of the milk, and the effect of the process upon flavor, keeping quality, pathogens, and vitamin A. H.P.

194. Safeguarding Your Water Supply. N. P. NUPSON, Pennsylvania Salt Mfg. Co., Philadelphia, Pa. Natl. Butter and Cheese Jour., *38*, 3: 42. March, 1947.

Bacterial contamination of the water supply may constitute a problem

of health or quality control or both, depending upon the types of organisms present. Get rid of improperly constructed wells, keep water supply tanks clean, and eliminate dead-end piping. If contamination exists, then the water coming in contact with the food can be pasteurized, the well itself may be chlorinated, the water supply tank can be chlorinated (usually 5 to 10 p.p.m. is adequate dosage), or a device can be used to inject hypochlorite solution continuously into the water line. W.V.P.

195. Saving and Disposal of Creamery Waste. O. W. SANDBORG, Armour & Co., AND A. J. STEFFEN, Wilson & Co., Chicago, Ill. Natl. Butter and Cheese Jour., *38*, 2: 34. Feb., 1947.

Improper disposal of dairy wastes can cause justified complaints and law suits. Dairy wastes are caused by inefficient equipment, methods, or operations. They may consist of buttermilk, skim milk, other by-products, rinsings, and wash water. The wastes can be reduced by using drippings from emptied cans for animal feed, using automatic controls where milk may overflow, preventing leaks, educating personnel to avoid spilling and splashing of products, and using all by-products. The system of waste disposal to be employed can be determined only by careful study of waste flow, waste concentration, proximity to a flowing stream, and relation to city sewage disposal. Frequent waste sampling and testing plus the determination of the causes of excessive losses are essential for loss reduction. W.V.P.

196. Booster Compressors. BERNARD SAVEY. Ice Cream Rev., *30*, 8: 52. March, 1947.

Booster compressors are suggested as the solution in adapting present ice cream refrigeration systems to fit the needs of the modern ice cream factory. Boosters are described as compressors which pump the gas from freezers, hardening rooms, and other low temperature rooms through water cooled and gas cooled coolers into the conventional or second stage compressors, which, in turn, compress and discharge the gas into condensers.

Advantages claimed for such a system are lower initial cost, maintenance of constant low temperatures unaffected by other plant loads, lower operating cost due to precooling of liquid and intercooling of low pressure gases, and less ammonia condenser cooling water required due to less total heat to be removed from the high pressure gas.

Although conventional type compressors may be used as boosters, boosters designed for this purpose are more economical. They are lighter in weight and have less bearing surface between the pistons and cylinder to contribute to friction losses. W.J.C.

197. Refrigerating Oil Carryover at High Temperatures. J. M. Lebeaux, and Luis H. Bartlett, Univ. of Texas. Refrig. Engin., *53*, 3 : 203–207. March, 1947.

Experimental results on oil carryover were obtained for 15 oil samples by means of a specially designed test flask. It was proved conclusively that refrigeration oils vaporize at the discharge temperatures of the compressor. The amount of oil carryover increased considerably with an increase in temperature, the pressure being held approximately constant. The greatest carryover occurred with most of the lighter oils. However, the heavier oils with lower carryover are unsuited to small high-speed installations. On the other hand, if the oil can be excluded from the evaporator, the pour test is a matter of little consequence. The oil also should have either a negligible solubility or complete solubility in the refrigerant. The most suitable refrigerating lubricants are petroleum oils of paraffinic and napthenic types; the latter, due to their inherent freedom from wax and resultant low pour test, are in general use throughout the refrigerating industry. The napthenic oils are mandatory where sulfur dioxide is used as a refrigerant but are equally satisfactory with the other common refrigerants. Heavier oils are called for in large machines for the heavier duty requirements or for refrigerants that have a high miscibility or mutual solubility with oil. Rotary type compressors require heavier oils than reciprocating or piston type.

The precise selection of the best lubricating oil will depend upon the design, capacity, operating conditions, and the type of the refrigerant used, and should be governed also by the vapor pressure of the oil to insure a minimum carryover and hence a maximum efficiency of the system.

<div align="right">L.M.D.</div>

198. Air Blast Quick Freezing. Regis Gubser, California Consumers Corp. Refrig. Engin., *53*, 1 : 23. Jan., 1947.

The essential features of air blast freezing systems are presented briefly. Air movement at the rate of 1500 c.f.m. per ton of refrigeration application is required. The maximum performance of a freezing tunnel is obtained when the air passages are kept small around the product and around the coil surface. The higher the velocity the greater the K factor. In no case should the velocity be less than 500 ft./min., and it should be as much above this rate as will be permitted by the friction loss and the static pressure developed by the fan.

<div align="right">L.M.D.</div>

199. Frozen Cooked Foods. Faith Fenton, State College of Home Economics and School of Nutrition, Cornell University. Refrig. Engin., *53*, 2 : 107–111. Feb., 1947.

Details of carefully controlled laboratory procedures for freezing certain

foods, including custard-base ice creams, are reported. Results favored the freezing of raw yeast rolls and unbaked pies and their subsequent baking after removal from freezer storage. Rolls made with milk scored higher in flavor and moisture than did those made with water. Ice creams frozen in a hand freezer and hardened in the various parts of the freezer cabinet were superior in smoothness. Ice cream frozen in the ice cube compartment, which ranged from 13 to 15° F., was smoother than that frozen in the freezer compartment, $-20°$ F., or in the storage space, $-5°$ F., the latter producing the coarser ice crystallization. The quicker freezing in the ice cube section, with the bottom of the tray containing the ice cream being in direct contact with the freezing plate, indicated that the rate of freezing was more important than the environmental temperature, even with forced convection in the freezer compartment. Ice creams developing coarse ice crystallization while freezing became increasingly coarse with prolonged storage.   L.M.D.

200. **A Qualitative Method for Detecting Surface Active Agents.** L. F. HOYT, National Aniline Div., Allred Chemical and Dye Corp., Buffalo, N. Y. Jour. Amer. Oil Chemists Soc., *24*, 2: 54. Feb., 1947.

A new method, consisting of the solubilizing of a Brilliant Oil Blue B.M.A. solution to produce a blue solution, is described for the qualitative detection of small amounts of surface active agents. The method is applicable to all types of surface active agents (*i.e.*, anionic, cationic, and nonionic) and to dry, liquid, or paste surfaces. Fifty agents have been tested.
                                                    J.L.H.

201. **World Food Outlook and the Dairy Industry.** GOVE HAMBIDGE, Canad. Dairy and Ice Cream Jour., *26*, 3: 25. March, 1947.

The work of the Food and Agricultural Organization of the United Nations is solving the widespread food shortage on a health standard for all people. The article discusses the dangers of over-production, the world food board, the Bruce commission to study proposals and make recommendations to the Food and Agricultural Organization, the high increase in production, and markets in backward lands.   H.P.

202. **Public Relations.** J. W. LAWRENCE. Canad. Dairy and Ice Cream Jour., *26*, 3: 33–36. March, 1947.

Public relations begin at home, and your own staff must be sold on the company before you can sell the public on it. The employees are partners as well as consumers in the industry. The producers' viewpoints must be recognized. Individuals and companies should work with their competitors

on the same major problems.   The dairy, in general, works for the interests of the public, and consumer education should not be neglected.   Profits and wages are the lubricant of industry.   Publicity is only one of the tools of public relations.   H.P.

203. The Need for Apprentice Training in the Dairy Industry.   W. H.
         SPROULE.   Canad. Dairy and Ice Cream Jour., *26*, 2: 28–30.   Feb.,
         1947.

A well-formulated apprenticeship scheme can aid greatly in developing trained workers to provide leadership to the industry in the years to come.
                                                                                    H.P.